MALARIA IN THE POST-WAR ERA[1]

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(Received for publication 13 November 1945)

Members of the National Malaria Society and guests—

I deeply appreciate the signal honor you have conferred upon me by electing me President of the National Malaria Society. At this time, when rapid strides are being made in the control of all insect-borne diseases, great advances in methods of controlling malaria infections are to be looked for in the near future. Indeed, the whole malaria picture as we return to peacetime pursuits, presents a greatly changed perspective. Let me discuss briefly malaria and its control as we view the problem today.

First. Malaria in the United States has undergone cyclic changes in severity and geographical distribution for the past forty years. When the available data are examined carefully it is evident that the gross changes from 1912 to 1935 are not striking; by way of contrast, however, malaria has undergone a steady and progressive reduction since 1935 which is entirely out of step with previous fluctuations. For the past two or three years, malaria has remained at an unprecedentedly low level in this country, and we may well ask, "Can further reduction be expected with the control measures now at hand?" We cannot say at this time whether malaria will remain at its present level but we believe there is a good probability that it will do so or, perhaps, undergo further reduction.

The reasons for this present low level of malaria prevalence are not very well understood but it probably results, in part at least, from the large number of major drainage outlets constructed during the 1930's.

Second. During the next two or three years we may expect malaria cases to be introduced into every part of the United States as a result of demobilization of the armed forces. It has already been demonstrated that American *anopheles* are capable of transmitting the foreign strains of plasmodia. Malaria- transmitting mosquitos exist in practically every State in the Union and it remains to be seen whether with a stage so well set local epidemics of malaria will develop.

I personally do not share the view of the alarmists in this connection. Most assuredly the possibility of new malaria foci can-

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not be overlooked, and should epidemics develop I believe we should plan to have at hand the organization, machinery, and knowledge to promptly isolate and reduce them.

Three. As a result of the war it is probable that the interests of Americans in the business world outside the United States will undergo great expansion. We can profitably broaden our horizons considerably and devote more attention to malaria and its prevention in other lands and also to practical methods of preventing the introduction of exotic strains into the United States.

Four. As a part of post-war industrial development extensive plans have been formulated for the creation of many water impoundments throughout the nation. The chief malaria vector in the United States is *A. quadrimaculatus,* an impounded water breeder, and by impounded water I refer to any still water from a water-filled hoofprint to a power lake. We all know of the explosive type of malaria outbreak that sometimes follows the impounding of water even in areas where malaria has not been considered a serious threat to the health of the community. It is my belief that these proposed impoundments will provide many new mosquito control problems wherever they may be located. As these projects represent industrial development, their construction is to be encouraged. Malaria is only one of the problems connected with impoundments and it is hoped that the spirit of mutual cooperation which has been developed between the builders and operators of these projects and the health authorities will continue, for only by a real understanding of each other's problems can desired results be realized in the mosquito control field.

Five. The 1941 Annual Report of the International Health Division of the Rockefeller Foundation stated: "The main obstacles in the way of malaria control are:

a. Fundamental absence of educated and effective public opinion.
b. Inadequate knowledge of methods for applying practically the results of research."

A little more bluntly stated this might be translated to mean—We know *how* to control malaria but have failed to educate the public as to the necessity for control and the methods employed.

We lack a sound long-term educational program to instill in the rising generation the value and means of preventing insect-borne diseases. This knowledge becomes of relatively increasing importance as the practice of modern sanitation causes these diseases to recede progressively to new low levels. Perhaps the results of an educational program among high school students introduced in a

Memphis High School a number of years ago by Mr. J. A. LePrince might give some interesting leads as to ways and means of developing an effective educational program.

We are frequently reminded of the possibility of eradicating malaria in the United States now that it is at a low ebb. I feel this is an untenable concept as we do not yet know in sufficient detail just where and under what conditions the disease occurs or will occur in its last natural habitat. Possibly malaria will be eliminated, but I much prefer to entertain the hope that we will build malaria out in our future developments and that we will attempt to "reduce" rather than "eliminate" it in its existing natural setting.

Constituted health authorities derive their funds mainly thru taxation and it is unwise for them to put malaria control operations into practice unless the disease is causing a measurable economic loss and unless the cost is in a measure commensurate with the economic ability of the people to pay. Viewed in this light it seems quite evident that we must devise more efficient and less costly methods of attack. It seems quite logical that for some time to come we could profitably continue to devote our efforts to two main lines of endeavor: (1) A program of pure research to open up new and more efficient avenues of approach, and (2) a program of applied research wherein new discoveries are implemented toward practical application in the field.

In further pursuing this view it may be well to enumerate some desirable lines of endeavor. These are not necessarily arranged in order of their importance:

1. There is a pressing need for more efficient drugs for the treatment and prophylaxis of the disease. "During the last four years the Office of Scientific Research and Development with the cooperation of the Army, Navy and Public Health Service has carried on a large and well integrated program aimed at discovering (1) a truly prophylatic drug of low toxicity, (2) a better suppressive than either atabrine or quinine, and (3) a truly curative drug especially against vivax malaria." (Quoted from a personal communication received October, 1945, from Dr. G. Robert Coatney, National Institute of Health,) When the full results of this program are released, we may find that much has been accomplished towards these ends. It has recently been demonstrated that atabrine will cure falciparum malaria (Fairley, 1945).

2. The possibility of producing a vaccine against malaria should be thoroughly investigated. People in rural areas have become accustomed to this method of disease control and it is believed

no great difficulties would be encountered in using a vaccine in either civil or military populations.

3. With the present low prevalence, the usual means of measuring malaria by blood smears, spleen surveys, or case histories have become unreliable if, indeed, not entirely worthless. We need a more accurate method of measuring malaria prevalence and one that will not produce undue antagonism when employed in areas where ignorance prevails.

4. Malaria prevalence is the result of a close partnership between man and the *Anopheles* mosquito. We need more information about the factors that permit infection in the mosquito to progress to the sporozoite stage. Also we need to know more about the limiting factors of mosquito reproduction and growth. Eyles (1943) evolved a simple method of estimating *anopheles* density within an area and we need to know the relation, if any, of *anopheles* density to malaria prevalence as a further check on the transmission of the disease. The reasons why *anopheles* are produced in certain waters and not in others where conditions seem to be identical have never been satisfactorily ascertained. Certain factors lead us to believe that perhaps *A. quadrimaculatus* is not a homogenous species. The entomologists should not permit this question to remain unsettled. Countless other problems involving the mosquito can be readily visualized.

5. In connection with the creation and management of impounded waters, new and very serious mosquito problems may arise for which engineers and entomologists must seek the answers. Many of these proposed impoundments will of necessity have a different fluctuation cycle from those customarily employed in the malaria belt. In many of these impoundments the mosquito control programs so admirably and carefully worked out for the Tennessee Valley Authority lakes can be made to apply only with major modifications. I feel that these problems will tax the resourcefulness and ingenuity of engineers and entomologists and we might as well prepare ourselves so that we will be in a position to suggest workable methods of minimizing mosquito production on these projects.

6. Further experimentation is indicated in the field of permanent ditch linings, not so much in the building of permanent drainage structures as in their utilization in large integrated drainage projects. The use of closed or pipe drains for land reclamation and for drainage in clay soils has not been fully exploited. The initial cost of permanent drainage is still too high and it is felt that field research will enable this to be lowered.

7. The development of DDT into a satisfactory larvicide will

take perhaps five years of field study. We are free to admit that DDT may be dangerous where fish are concerned. On the other hand it is without doubt the most efficient larvicide yet discovered and it will eliminate mosquito breeding in places and under conditions where no other larvicide can be made effective. In a large scale seasonal test just completed the cost of larviciding a difficult area of drainage ditch was reduced 75 per cent by the use of DDT in oil as against the usual oil larvicide alone. We can, I am confident, by field work in practical applications produce a DDT larvicide which will reduce the present costs by at least 50 per cent and make larvaciding effective without interfering with wild life..

Against adult mosquitoes, DDT is a potent means of controlling the insects in buildings. The methods of utilizing DDT for this purpose have been reasonably well developed. It has been shown that an attack on the adult mosquito in the home offers the only known method of promptly ending malaria epidemics. As far as I am aware, an attack on adult mosquitoes as a means of controlling a malaria outbreak has never been made in this country. The method may become very important in the future.

This incomplete list serves to indicate the wide field in malaria research and control still open to the medical profession, engineers, entomologists, and other specialized professions.

The time seems to be at hand when the organizations and activities of the National Malaria Society need a careful review and probably some alterations to bring its activities in step with present malaria problems. A large increase in our membership has occurred during the past few years as a result of the increased number of persons working in this field; an increase largely due to malaria control by the armed forces. The Society today doubtless represents the greatest wealth of malaria control knowledge in the world and we should prepare to make use of this knowledge.

As operated at present, the whole membership of the Society is not represented as it should be. There was a time when a large percentage of the membership was represented at the annual meeting but this is no longer the case and it seems desirable that some means should be provided whereby the entire membership could have a voice in the affairs of the Society.

The creation of a governing council or other similar body to handle the business of the Society between meetings could operate to prepare certain agenda for forthcoming meetings and it could in some measure at least serve to represent the members at such meetings. Further, it appears desirable that the nominating committee should be appointed well in advance of the annual meeting so that

the membership could be properly canvassed for prospective officers. Our constitution needs revising to make it more adaptable to our present situation and I am glad to state that the constitution committee has worked diligently to effect such a revision and will present its recommendations at the business session.

I concur heartily in the statement made by Mr. O'Neill in his presidential address in 1942 to the effect that the whole question of standing committees is in need of critical review and that their functions should be redefined. It is quite evident that the work of these committees has deteriorated in past years possibly because of the type of action, or may we say lack of action taken on their reports. In my opinion the work of these committees should be a most important part of the Society's activities and at least some of the reports should become the subject of discussion at our business sessions. I have heard the opinion expressed by some members that the discussion of such reports at the annual meeting is of equal importance with the reading of papers inasmuch as the papers subsequently become available to the members through the Journal.

It is fully realized that changes in the operation of the Society should be brought about only through gradual changes after consideration of the available factors by a duly appointed group of the members. I therefore submit the above for the consideration of the Society.

In closing, let me say that I am confident that all workers in the malaria field are ultimately interested in malaria control and are looking forward in the hope that new and more effective methods of malaria control will shortly be developed along medical, engineering, and entomological lines. However, a word of warning is appropriate. Up to the present time if any practical inroads on malaria prevalence have been made they have been mainly along lines aimed at breaking the association between mosquito and man. The admonition of Dr. L. L. Williams quoted in Mr. Bradley's presidential address of last year to "Place your eye on the mosquito and keep it there" is still an appropriate one for us to follow until other means of control have proved superior to those heretofore employed.

References

Eyles, Don E.
1943. The measurement of a population of *Anopheles quadrimaculatus*, Say. Jour. Natl. Mal. Soc., 2:71-83.

Fairly, H. Hamilton
1945. Chemotherapeutic suppression and prophylaxis in malaria—an experimental investigation undertaken by medical research teams in Australia. Trans. Roy. Soc. Trop. Med. & Hyg., 38:355-365.

NOTES ON THE MORBIDITY OF NATURALLY OCCURRING MALARIA[1]

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(Received for Publication 28 May 1945.)

In connection with studies on mosquito proofing of homes, data on malaria morbidity were accumulated in 1940 in 3 areas of the Tennessee Valley and are of a character which permits their analysis in terms of duration of illness. The findings of this analysis appear to be of sufficient interest to merit inclusion in malaria literature, which, voluminous in most respects, contains relatively meager records of this type.

In the manner reported previously by Watson and Rice[2], morbidity data were collected by public health nurses who made weekly visits to all homes in the 3 areas from 6 May to 28 October 1940. The history of a febrile illness occurring in a family since the last visit of the nurse, the symptomatology of which suggested that it may have been due to malaria, was recorded as such. The date of onset of symptoms was noted and the subsequent duration of the illness was observed. These records and observations are the basis of the present report. Duration of malaria morbidity, for the purpose of this study, is defined as the period during which symptoms attributable to malaria infection occurred. Accordingly, some persons experienced several episodes of malaria illness during the observation period. In instances where more than one illness was recorded for the same person, a period of at least one week when no symptoms were reported intervened between the illnesses. No attempt has been made to separate illness due to relapses from that caused by initial attacks. Therefore, the initial illness and all subsequent malaria morbidity recorded for the same person may have been due to one, or more than one, infection.

An attempt was made to secure a blood film from each sick person. Accordingly, films were secured from 65 per cent of all recorded cases at some time within 3 weeks of the date of onset of illness. Only 25 per cent of the total blood films were positive for malaria parasites; but of the films taken on the date of onset of illness, approximately 50 per cent contained parasites.

During the period of observation there was no systematic treatment of malaria cases. Probably every case received some form of

[1] The studies on which this paper is based were made in cooperation with the Alabama State Department of Public Health.

[2] Watson, R. B. and Rice, M. E. 1941. Further observations on mosquito proofing for malaria control. Am. J. Hyg., 34 (Sec. C): 150-159

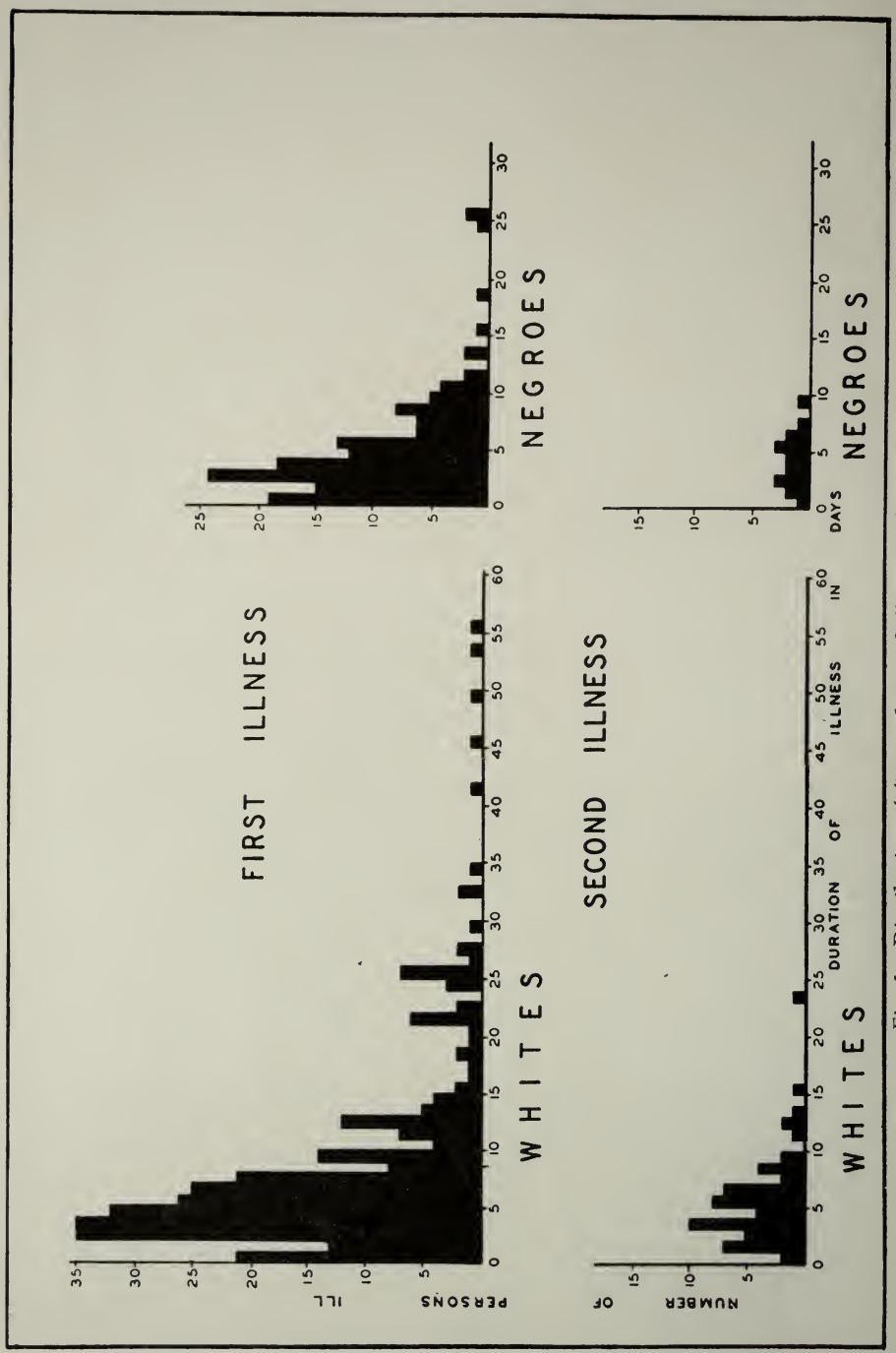

Fig. 1—Distribution of first and second illness by duration and race.

medication, rarely under the direction of a physician. This circumstance, together with the low economic level of the population, would appear to make the findings of this study applicable to many situations in the South.

The two racial components of the population under consideration were approximately equal, being composed of 1,058 white persons and 1,101 negroes.

The Duration of Malaria Illness

Table 1 presents the duration of all malaria illness, classified by race and sex for the persons who were under observation from 6 May through 28 October. It is to be noted particularly that the median duration of illness is longer among white persons than among negroes, 5.4 and 3.8 days per case respectively. A remarkably similar experience was noted for negro males and females (3.3 and 3.9) and for white males and females (5.2 and 5.6). The white persons not only experienced more days of illness from malaria than negroes, but they also suffered a larger number of attacks (0.37 and 0.17 cases per person per season respectively).

Table 1—Distribution of all Illness Attributed to Malaria by Duration, Sex and Race

Duration in days	Number of Cases of Malaria					
	White			Negro		
	Male	Female	Total	Male	Female	Total
1	13	12	25	8	12	20
2	14	7	21	10	8	18
3	22	19	41	14	14	28
4	25	22	47	6	14	20
5	27	10	37	8	8	16
6	16	18	34	5	12	17
7	20	13	33	1	7	8
8	12	11	23	3	4	7
9	9	3	12	5	3	8
10	10	6	16	1	5	6
11	3	1	4	2	2	4
12	3	5	8	1	1	2
13	4	11	15	–	–	–
14	2	5	7	1	1	2
15	2	2	4	–	–	–
16	2	1	3	1	–	1
17	1	–	1	–	–	–
18	1	–	1	–	–	–
19	2	–	2	–	1	1
20+	19	14	33	1	2	3
Und.	5	5	10	4	1	5
Total*	207	160	367	67	94	161
Days	1679	1346	3025	340	514	854
Median days per case	5.2	5.6	5.4	3.3	3.9	3.8

* Does not include 15 cases of undetermined duration

When the duration of illness is shown graphically (figure 1), its distribution is seen to be quite skew with regard to both first and second illnesses. No data are shown for later attacks since there were only 14 third and 2 fourth illnesses recorded.

Of the 49 illnesses which persisted for 15 days or more, 47 were first attacks and 2 were second attacks. Blood films were secured from 35 of these individuals, and in 9 films malaria parasites were found (7 *Plasmodium vivax* and 2 *P. falciparum*.) Of the 5 illnesses which persisted for six weeks or more, blood films positive for *P. vivax* were obtained in 3 cases and the other 2 were negative.

Table 2—Blood Films on Reported Cases of Malaria

	Whites			Negroes			Total		
Days after onset	Number* films	Per cent vivax	Per cent falciparum	Number* films	Per cent vivax	Per cent falciparum	Number* films	Per cent vivax	Per cent falciparum
0-10	184	19.0	1.1	102	23.5	5.9	286	20.6	2.8
11+	38	7.9	13.2	8	0.0	25.0	46	6.5	15.2

*23 films made after undetermined interval and 188 cases with no blood film not included.

It appears that *P. vivax* infections are more likely to be confirmed parasitologically if slides are taken within 10 days after the onset of the illness, while confirmation of *P. falciparum* infection is made more frequently after 10 days (table 2). While the numbers are small, the trend of this observation is the same for both races. Doubtless this is due in part to the fact that the gametocytes of *P. falciparum* begin to appear about 10 days after patency is established and they tend to persist in the blood stream in spite of treatment.

The Relation of Blood Survey Findings to the Occurrence and Duration of Malaria Morbidity

An attempt was made in table 3 to relate the findings of a blood film survey made between 1 and 15 October 1940 with the re-

Table 3—Ratio of Cases of Malaria to Positive Blood Films on October Survey, 1940

	Number of persons*	Number of cases	Average number cases per 100 persons	Blood films		Ratio (2)/(4)
				Number positive	Per cent positive	
	(1)	(2)	(3)	(4)	(5)	(6)
Whites	823	304	36.9	10	1.2	30.4
Negroes	750	126	16.8	41	5.5	3.1
Total	1573	430	27.3	51	3.2	8.4

*Persons under observation from 6 May to 28 October and who had blood films on October survey.

ported occurence during the summer of cases believed to be malaria. The persons included in Table 3 were under observation the entire time from 6 May to 28 October, and all of them had blood films during the October survey. It will be noted that among white persons about 30 cases of malaria were recorded for every positive blood film found; for negroes, about 3 cases; and for both races combined, about 8 cases.

A similar attempt is made in table 4 to relate the mean number of sick days experienced to parasitemia rates. Here the racial difference noted already is even more extreme; while negroes had 16 sick days for every positive blood film, white persons suffered no less than 254.

Table 4—Ratio of Days of Illness to Positive Blood Films on October Survey, 1940

	Number of persons*	Number of sick days	Average sick days per 100 persons	Blood films		Ratio (2)/(4)
				Number positive	Per cent positive	
	(1)	(2)	(3)	(4)	(5)	(6)
Whites	814	2544	312.5	10	1.2	254.4
Negroes	745	672	90.2	41	5.5	16.4
Total	1559	3216	206.3	51	3.3	63.1

*Persons under observation from 6 May to 28 October and who had blood films on October survey but not including persons who had illness of undetermined duration.

Discussion

The relative tolerance of negroes to infection with malaria parasites, as compared with white persons, has been noted frequently. In the population under consideration the parasitemia rate determined by survey is almost five times higher in negroes than in white persons. This circumstance is believed to be due in part at least to the fact that the negroes were relatively unprotected from malaria transmission. Less than half (48 per cent) lived in homes which were protected by mosquito-proofing, while more than three fourths (84 per cent) of the white population lived in mosquito-proofed homes. This circumstance almost certainly had some bearing on the total number of malaria infections experienced by the negro component of the total population.

The relative tolerance of negroes to malaria infections is emphasized in this study by the duration of both the first and second illnesses, and also by the number of attacks experienced. These variations are believed to reflect fundamental differences in the response of the two races to malaria parasitism. They may also reflect a racial difference in attitude toward the discomforts produced

by malaria illness. It is our impression that in the population studied, negroes were less likely than whites to complain about anything.

When the number of cases of malaria experienced during the entire season is related to the occurrence of positive blood films in October, the racial difference is even more marked. Apparently the October surveys give a fairly good idea of the amount of malaria experienced by the negro component of the population during the season, but they underestimate badly the amount of malaria illness suffered by the white component.

The question arises naturally as to the credence which can be placed on reports of malaria morbidity, unconfirmed parasitologically, received from the white persons in this study. Doubtless these reports should be discounted to some extent. The much larger number of illnesses of 10 days or longer among white people, as compared with negroes, (table 1) supports this point of view. However, the extent to which the reports should be discounted cannot be determined on a factual basis. There is a tendency for persons living in a malarious place to ascribe all illness to malaria infection and to practice self-medication. This fact and the influence of treatment are variables which are almost impossible to appraise.

Summary

A population of 2,159 persons made up of approximately equal negro and white racial components was kept under observation from 6 May to 28 October 1940. A weekly visit was made to each family to determine whether any member was ill with malaria at the time of the visit. Also, histories of illness occurring in each family since the last visit were appraised in terms of the probability of their having been due to malaria. The duration of each reported case of malaria was recorded and blood films were obtained whenever possible from every case. Finally a blood film survey was made of the entire population in October.

Data are presented which appear to show that under similar or worse environmental circumstances negroes suffer less from malaria morbidity than white persons, with regard to both total number of cases and duration of illness, although it was determined by blood film surveys that the negroes had a greater relative number of infections.

AN APPRAISAL OF THE MALARIA ENDEMIC IN PROTECTED AND COMPARISON AREAS OF SARDINIA IN THE YEARS 1925-1934*

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(Received for publication 9 October 1945)

In March 1925 antilarval measures were begun in Portotorres, a town of over 6,000 persons on the northwestern coast of Sardinia. The project was the first to be initiated by the newly created Malaria Experiment Station in Italy under the direction of Professor Missiroli. The Station was a collaborative undertaking of the Italian Government, through its Department of Health, and The Rockefeller Foundation. Participation by the Foundation ceased at the close of 1934, and this appraisal is limited to the period from 1925 through 1934.

At the time the project was initiated no one in Italy seriously believed in the possibility of controlling malaria through antimosquito measures alone. Grassi's use of them in the neighborhood of Fiumicino in the Roman Campagna had not successfully reduced the disease. The Italian Government was engaged in large-scale drainage projects, some of which reduced the amount of malaria while others actually created new breeding places of anophelines as productive as those eliminated. At this time the Pontine Marshes had not been drained, and in villages on their margins the population was virtually 100 per cent malarious.

The Government's chief antimalaria measure was the treatment of the sick with quinine in free dispensaries throughout the malarious areas. Local landowners carried part of the expense by paying for the quinine. A primary requirement for all the projects of the Malaria Experiment Station, therefore, was that the antilarval measures should not exceed in cost the amount necessary for the routine treatment programs. This proviso should be stressed at the outset. The object of the undertaking was not complete eradication of a species of anophelines or, necessarily, the elimination of malaria from the area. The purpose was to see what a limited program would accomplish in a highly malarious community.

The work of the Malaria Experiment Station in the early years (1925-1928) has been discussed by one of us (Hackett, 1929). When operations began in Portotorres in Sardinia, the parasite rate

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation and the Italian Department of Health.

Fig. 1—Map of Sardinia.

was 34 per cent and the spleen rate 47 per cent among children from 1 to 12 years of age. In 1924 the dispensary case rate was 213 per 1000 population, and in 1925 the infant infection rate was 3.8 per 100 births. The source of anopheles breeding was a sluggish river, 12 kilometers of which were within easy flight range of the town, three or four marshes formed by the river or its tributaries, and miscellaneous collections of standing water. Anophelines present were *Anopheles labranchiae labranchiae* and a rare *A. sacharovi,* both carriers, and *A. algerinesis* which could be ignored. It was found that the most effective antilarval measure as well as the cheapest was the application of Paris green. An area extending somewhat over 3 kilometers from the center of the town was eventually covered. Application of Paris green was begun in late March or early April of each year and continued through September.

Antilarval measures were not the only ones employed against malaria in the communities to be considered. Local dispensaries continued to treat the sick who applied for quinine. Furthermore certain experiments in the intensive use of quinine were conducted by the Malaria Experiment Station. These were later discontinued in Portotorres, but the towns of Torpé and Posada in northeastern Sardinia were the scene of intensive measures to reduce transmission by the use of quinine, plasmoquine, and atabrine. No antilarval activities were carried on in these two towns or in Lodé, which was observed merely as a statistical comparison area.

The town of Siniscola was selected in 1927 to serve as a comparison area for Portotorres since conditions affecting the malaria endemic were similar. During the first year of observation, however, the authorities brought pressure to bear, with the result that antilarval measures were instituted in 1928 in an area extending 3 kilometers from the town.

The accompanying map of Sardinia (Figure 1) shows the location of the towns included in this review.

The object of this paper is to examine the data collected during the period of the project in Portotorres and in four other Sardinian towns having similar problems and to describe by simple statistical methods the malaria endemic during the interval covered. Our reason for doing this is (1) to so present the data that the time changes and the comparisons between areas may be shown to the best advantage, (2) to bring out the relationships between the various measures of malaria prevalence, and (3) to establish simple statistical techniques which may be employed in the evaluation of data similarly collected for other projects.

Material for analysis

A wealth of data was collected during the studies in these five towns. The problem has been to select those best suited to bring out the pertinent aspects of the endemics. The following indexes and rates describing the prevalence of malaria have been employed:

1. *Captures of adult anophelines.* Capture stations were designated in four of the five communities. None were set up in Lodé. Weekly visits were made to each station and a record was kept of the number of adult anophelines captured. Procedures were similar in each area, but the work was done by different inspectors. Since the number of weekly visits to each station differed, comparisons of density between areas must be made with captures on a visit basis. It must be assumed that the level of the density index in one area is comparable with that of another, in that a similar proportion of the anopheles population was routinely captured in each. It must also be assumed that time changes in the index were similar to those occurring in the anopheles population in the area.

2. *Malaria case rate.* The number of persons treated in the dispensary does not furnish a complete record of the sick persons in the community. Furthermore, since the rate includes persons having relapses, it does not represent new infections. The data are available, however, and may be examined.

3. *Infant infection rate.* This is a rate of recent transmissions. Information on infection among infants under 1 year of age was obtained by a nurse who made house to house visits. The rate was based on the results of blood examinations of ailing babies and is available for Portotorres and for Siniscola only.

4. *Parasite rate.* Surveys of children were made each year (January through March) to determine the presence of parasites.

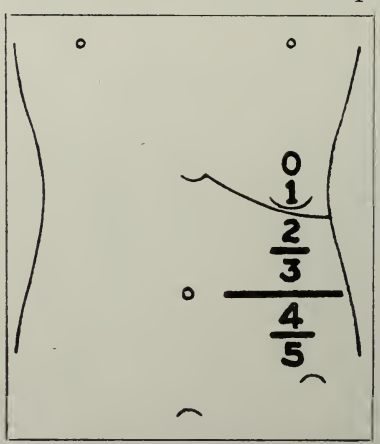

Fig. 2—Diagram showing categories of spleen enlargement.

Children from 1 to 12 years of age received blood examinations; they were presumably well at the time. The parasite rate was based on the examination of thick drops stained *en masse* with Giemsa in a tank, according to Barber's technique. The search for parasites was limited to 3 minutes.

5. *Spleen rate.* This measure was obtained from children who were examined in the winter survey. Spleens which extended below the costal margin, with or without deep inspiration, were considered enlarged and entered into the computation of the rate.

6. *Average spleen.* The method of classifying and analyzing spleens as to size has been discussed recently by one of us (Hackett, 1944). The extent of the advance of the spleen with respect to the costal margin was entered under one of the categories described below. The area delimited by these classes is shown in Figure 2:

0 = normal or non-palpated spleen ("negative" spleen).
1 = spleen palpable only when the subject draws a deep breath.
2 = spleens ranging from those at the costal margin palpable without assistance from the subject to those whose lower border reaches a point halfway to a horizontal line through the umbilicus.
3 = spleen projecting more than halfway to the umbilical level but not beyond it.
4 = spleens below the umbilical level but not more than halfway to the line of the symphysis pubis.
5 = all spleens larger than those mentioned above.

From the frequency distributions of spleens set up for each survey an average or mean was computed with categories numbered as above. This centering value gives a rough measure of the average size of spleen in a given population. It is a more sensitive measure of malaria prevalence than the spleen rate, since it takes into account both the recession of the spleens from larger to smaller categories and the increasing proportion of children with normal spleens.

7. *Average enlarged spleen.* This is really the average size of spleens that are enlarged, without regard to those that are or that become normal. It, too, should decline as the amount of malaria transmission is reduced but not as rapidly as the average spleen. As the number of persons with enlarged spleens who pass into the normal group increases the number left in the enlarged spleen group automatically decreases so that this index is derived from fewer and fewer persons as the malaria endemic subsides.

8. *Average spleen in relation to the presence of parasites.* Since the spleen and parasite examinations were made at the same

time the results could be cross-tabulated, as shown below, so that the average spleen for children with parasites and that for children with no parasites could be computed. Both indexes are important ones to watch. The first applies to known infected individuals and is derived from a decreasing number of persons as the endemic recedes. The average spleen for children with no parasites is derived from those who are uninfected, the number of whom increases as time passes, plus those retaining some enlargement but showing no parasites.

Cross Tabulation of Results of Spleen and Parasite Examinations
Siniscola (March 1930)

	Spleen categories				Total
Parasites	0	1	2	3	
Present	8	18	16	4	46
Absent	196	62	27	5	290
Total	204	80	43	9	336

Methods of analysis

Of primary importance in the analysis of these data is a proper method of graphic presentation. This applies to charts showing anopheles density and case rates on weekly or monthly basis as well as to the trends of annual rates and indexes. Since the production of both anophelines and plasmodia is multiplicative, a logarithmic scale should be employed. Such a scale brings out relative differences, so that similar rates of rise or fall plot as parallel lines regardless of the actual level of the particular measure employed. The significance of this will be apparent when illustrations are given.

Because of this geometric character of the data, straight lines were fitted by least squares to the logarithms of the yearly rates and indexes. The formula for this line is:

$$y = ab^x \text{ which may be written}$$
$$\log y = \log a + x (\log b)$$

in which y = the computed rate or index
and x = the interval in years

The regression coefficient (b constant) indicates the annual per cent or rate of change, and a comparison of these constants will show which series of indexes is decreasing most rapidly. The a constant furnishes a measure of level. In this analysis it is the point on the fitted line one year prior to the beginning of the period shown.

Variance analysis described by Snedecor (1934) has been used for comparing means of subgroups with that of the total. Chi square tests of Fisher (1944) have been applied to fourfold tables to determine the presence of association between two variables. They have also been used to determine whether two or more frequency distributions came from the same parent population.

Data for Portotorres will be presented for the entire period of

observation, 1925-1934. Those for Siniscola will be limited to the years 1929-1934, except for the comparison of dispensary case rates with those of Portotorres in 1927. Since no antilarval work was ever done in the towns of Lodé, Posada, and Torpé and since their initial spleen and parasite rates were similar, data for these three towns have been combined to form a comparison area. The period covered was limited to the years 1929-1934 for two reasons: (1) The method of recording enlarged spleens was changed in 1929, so that comparisons could not easily be made with earlier records. (2) Also the results of spleen and parasite examinations were not cross-tabulated in the earlier years.

Results

Anopheles prevalence and the dispensary case rate. Both Portotorres and Siniscola are situated near rivers which, together with adjacent marshes, were sources of heavy anopheles breeding. Spleen rates prior to the beginning of antilarval work were: Portotorres 47 per cent (1925), Siniscola 56 per cent (1927). Parasite rates were 34 and 19 per cent respectively. This was the third season of antilarval activities in Portotorres, while no significant work was done in Siniscola until 1928. Observations in Siniscola began in March 1927. Figure 3 compares the monthly malaria case rates in each town during 1927 as well as those for Portotorres in 1924, the year prior to the initiation of antilarval work. The frequency of cases in Siniscola was at a high level throughout the year and well above that for Portotorres in either year. Differences were relatively greater early in the year when spring relapses occurred

A comparison of the seasonal wave of anopheles density for Portotorres with that of malaria patients in the dispensary during the months of May through September in 1930 and 1931 is made in Figure 4. The value of the logarithmic scale is apparent here. The similarity in the shape of the summer epidemic of malaria infection to that of the wave of anophelines in each season is apparent notwithstanding the different levels. The timing of the epidemic with respect to the influx of anophelines is well shown. In both years an interval of seven weeks occurred between the initial rise in density and the corresponding rise in dispensary attendance.

This period of 49 days may be of significance. It furnishes two 12-day intervals in which anophelines that bite infected persons may become infective, and two additional 12-day periods during which persons bitten by infected mosquitoes may develop their initial attacks. Only vivax infected persons may become infective to anophelines within an average interval of 12 days, since 20 days must elapse before gametocytes of *P. falciparum* appear in the peripheral

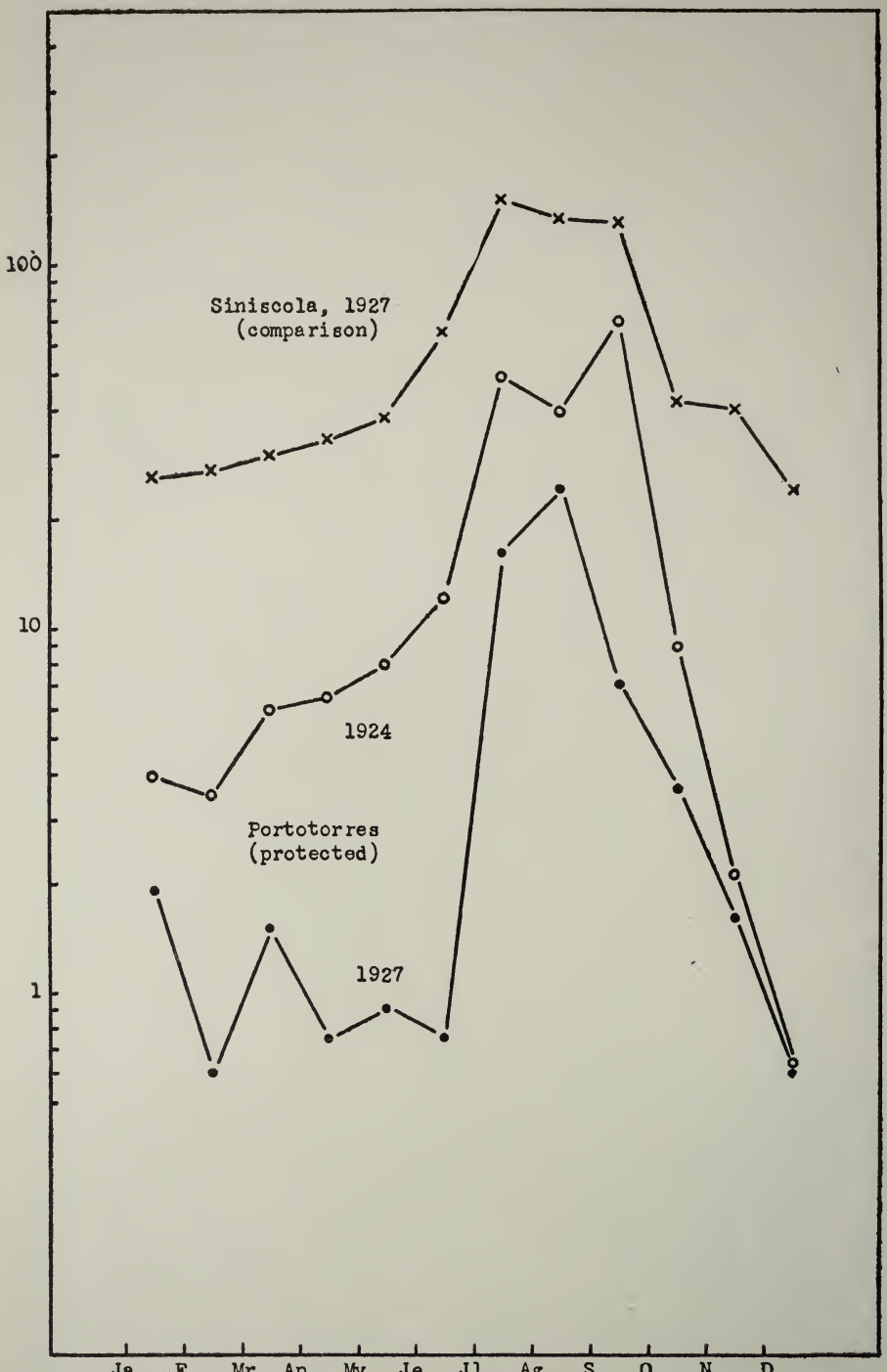

Fig. 3—A comparison of monthly dispensary case rates in Siniscola in 1927 with those of Portotorres in 1924 and 1927.

blood (Kitchen and Putnam, 1942). Thus the 49-day interval provides for two complete vivax transmission cycles for the initiation of the seasonal epidemic.

A comparison of seasonal fluctuations of anopheles density with those of malaria infections was also made in a paper by Howard, Earle, and Muench (1935) in which data on adult captures of anophelines and on malaria infections from protected and comparison zones in Puerto Rico were used for analysis.

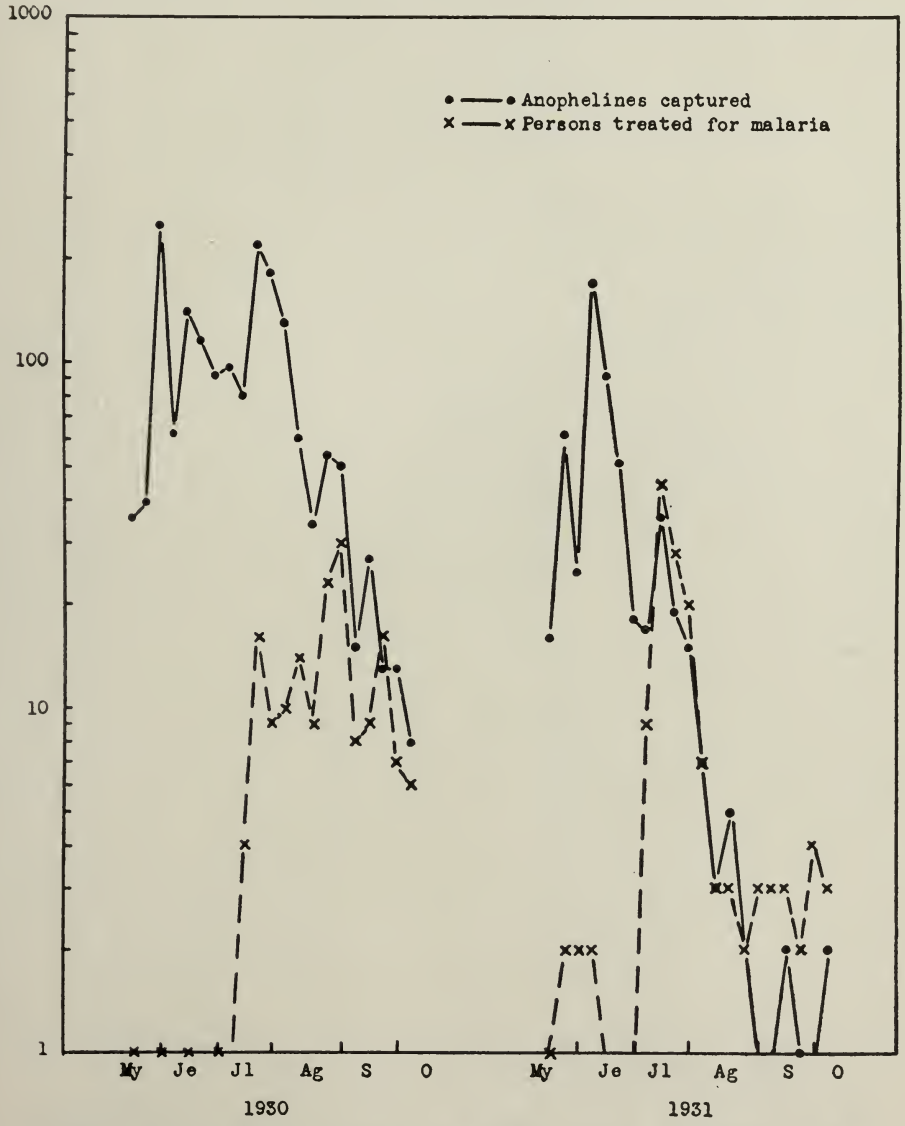

Fig. 4—Partotorres—A comparison of weekly captures of anophelines in 5 stations with persons treated for malaria in the dispensary, May—October, 1931.

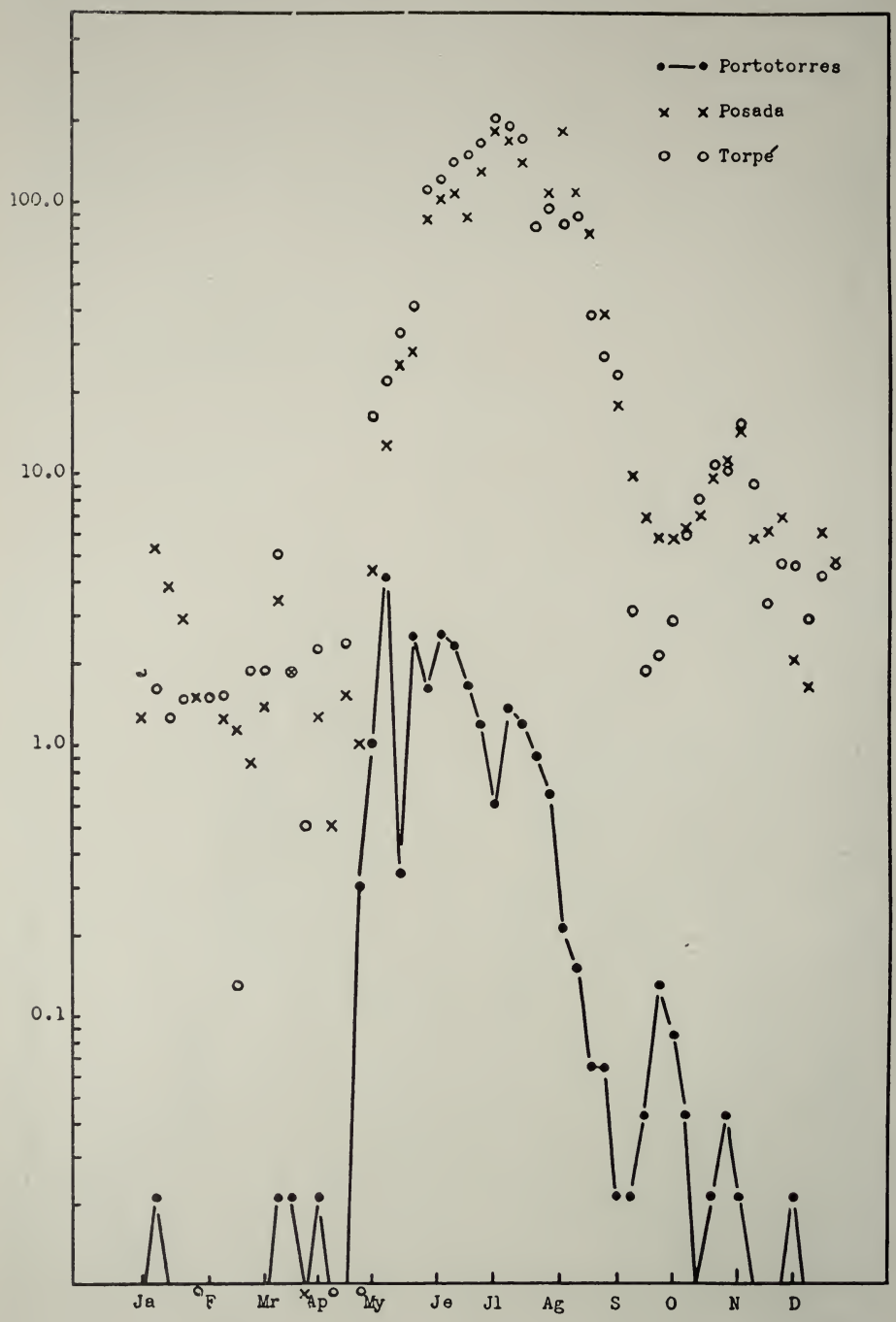

Fig. 5—A comparison of anophelines captured weekly in Portotorres (protected) and in Posada and Torpé (comparison) in 1932.

Finally, Figures 5 and 6 show the weekly captures of anophelines per visit in Portotorres and in the two comparison towns of Posada and Torpé for the years 1932 and 1933. The season 1932 was obviously one of prolific anopheles production in the two comparison towns, and the difference in peak density between these areas and Portotorres was great. Density at the beginning and end

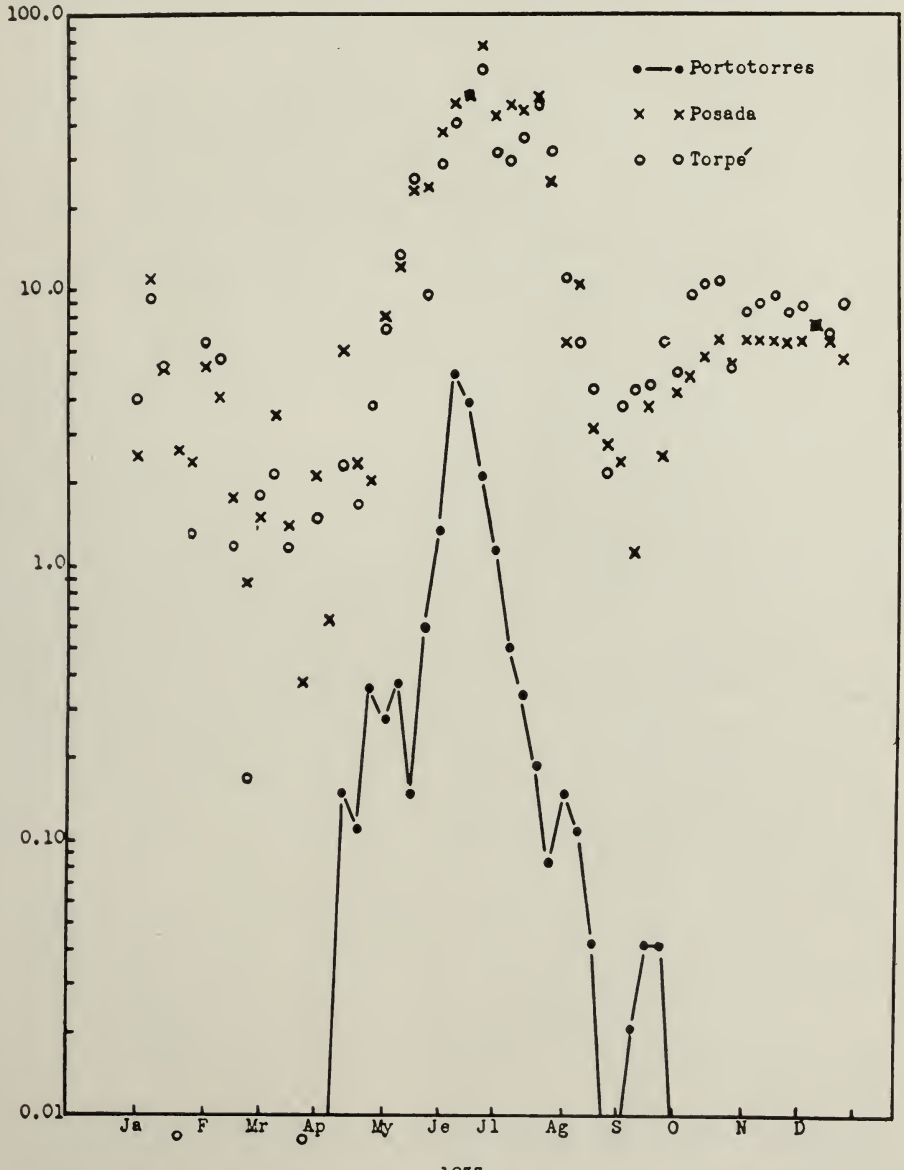

Fig. 6—A comparison of anophelines captured weekly in Portotorres (protected) and in Posada and Torpé (comparison) in 1933.

of the year in the unprotected towns was maintained at a high level in both years. In Portotorres, on the other hand, there were many weeks at either end of the season when no anophelines were captured. Protection was almost complete in Siniscola by this time. Ten anophelines constituted the largest catch of any week during 1932 and 1933.

Enough has been said to show the changes in anopheles density in Portotorres during the period of observation. In spite of the 3 kilometer zone of antilarval activity, anophelines always invaded the town during the summer, but they were frequently not found in the spring or fall. In Siniscola anophelines were virtually absent. In Posada and Torpé, where no antilarval measures were employed fluctuations in density were dependent upon rainfall and other climatic factors. The malaria epidemic indicated by persons attending the dispensary in Portotorres resembled in form the wave of anopheles density observed approximately 7 weeks earlier.

Time changes in the malaria endemic in Portotorres. A picture of the time changes in the amount of malaria during the period of antilarval operations in Portotorres can best be obtained through an examination of the various rates of malaria prevalence set up on an annual basis. These are shown in Figure 7 and are compared with anophelines captured during each year in terms of the number of visits made weekly to stations within the protected zone. Since the vertical scale of the figure is logarithmic, similar relative changes give parallel lines.

The first impression one gains from an inspection of the figure is that the rate of change indicated by the downward sweep of the indexes was not constant. The drop was sharp and on the whole rectilinear from 1925 through 1929. In the years 1929-1934 there were sharp fluctuations with no overall decrease. Following the initial downward plunge, captures of anophelines rose sharply in 1930 and again in 1932 before continuing their descent. The malaria case rate dropped less sharply in the years 1925-1929. It, too, rose in 1930 and again sharply in 1934. The infant infection rate dropped as rapidly as the case rate during the early years and rose sharply in 1930 and 1931. The last five rates are based on infections in 4, 4, 1, 1, 1 infants, so differences are more apparent than real. The essential parallelism of the trends of these three measures is important, however.

Straight lines have been fitted to the logarithms of the spleen and parasite rates derived from the winter surveys of children from 1 to 12 years of age, although the trends were not rectilinear they were similar to that of the case rate. Since the surveys were made at

the beginning of the year indicated, the rates really measure the amount of malaria carried over from the previous transmission season. No survey was recorded for 1935, so we do not have spleen and parasite rates for the season giving rise to the case rates in 1934.

The annual rate of decrease is given by the regression coef-

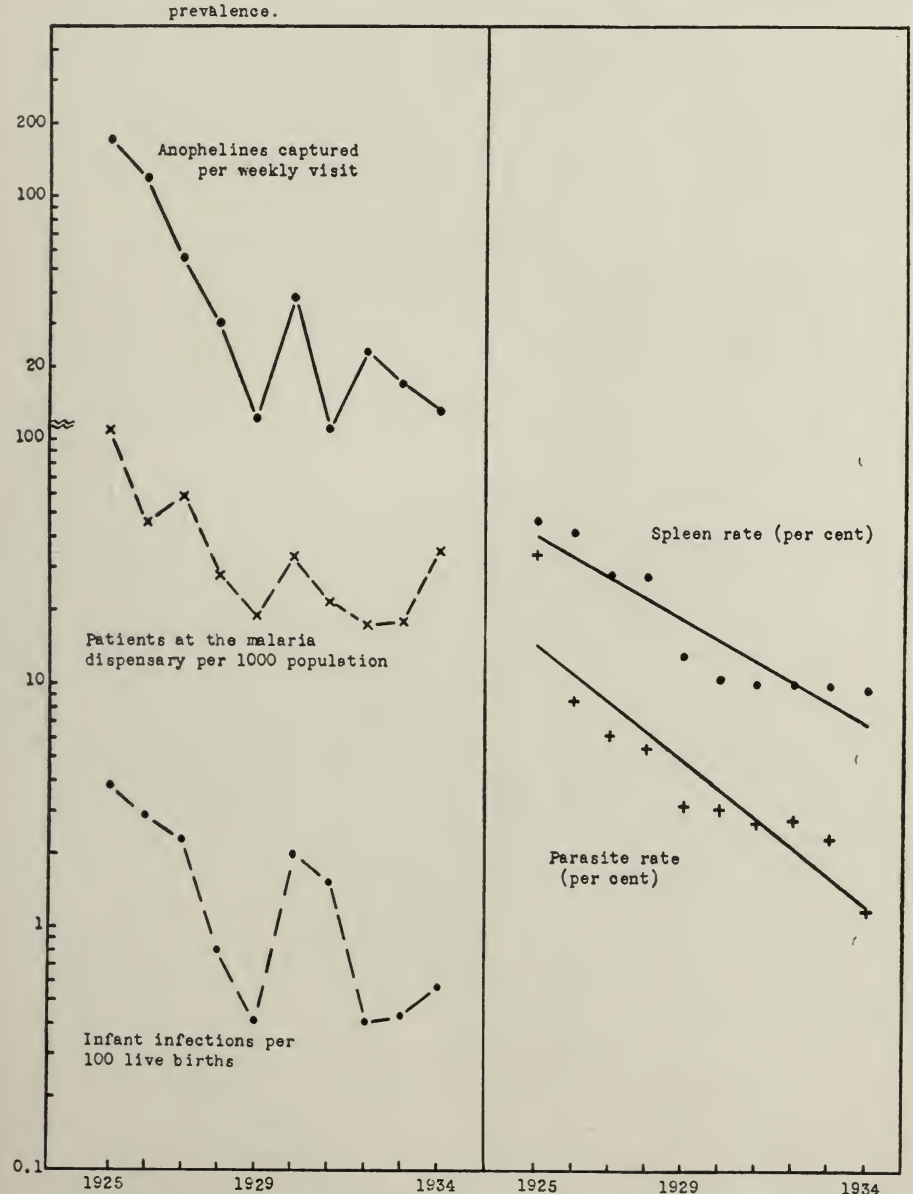

Fig. 7—Portotorres, Sardinia. The time trend of anophelines captured in the unprotected zone compared with that of various measures of malaria prevalance.

ficient (b constant) obtained when the line is fitted. Those computed for the lines plotted in Figure 7 are as follows:

Regression coefficient (b constant)

	Logarithm with standard error[1]	Annual rate of decrease Per cent
Spleen rate	$-0.086 = 0.012$	18.0
Parasite rate	$-0.119 = 0.020$	24.0

Each logarithm is more than twice its standard error, so that the rates of decrease are significant. The difference between the two logarithms (0.033 ± 0.023) is not equal to twice its standard error, however, so the decrease of the parasite rates cannot be considered significantly greater than that of the spleen rates.

In reviewing the accomplishments in the Portotorres area we may say that during the period of antilarval measures anopheles density dropped to a figure less than one-tenth that observed at the beginning of the period. The annual rate of decrease was less rapid for the malaria case and spleen rates but was apparently equally rapid for parasite rates. Annual fluctuations were similar and leave the impression that changes in anopheles density affected the disease prevalence.

The a constant gives the theoretical value for the particular rate or average considered which lies on the fitted line one year prior to the beginning of the period shown in the figure, in this instance 1928. These constants are similar to geometric means which are computed from the logarithms of the observed measures without regard to their slope.

Malaria prevalence in protected and in comparison areas

The comparison of the malaria endemics in the protected towns of Portotorres and Siniscola with those in the towns of Lodé, Posada and Torpé, where no antilarval work was done, is limited to the years 1929 through 1934 and is based on the measures of residual malaria derived from the winter surveys of children from 1 to 12 years of age. These were the years when malaria in Portotorres showed little decline as indicated in Figure 7. It will be of interest, however, to compare its level with that in the other areas. This can be done by examining Figure 8, in which the trends of spleen and parasite rates and of average spleens and average enlarged spleens are shown. Tables 2 and 4 contain the specific rates and averages, and the original data are given in Table 3. Table 1 contains the constants

[1]Standard error $_b = \left\{ \frac{(\Sigma \text{ log observed—log calculated values})^2}{(n-2)\left\{ \Sigma X^2 - \frac{(\Sigma X)^2}{n} \right\}} \right\}^{\frac{1}{2}}$

for the fitted regression lines shown in Figure 8. We shall discuss first the difference in level (*a* constants) and second the differences in slope (*b* constants).

The level of spleen and parasite rates as well as that of the average spleen indicated by the *a* constants was significantly lower for Portotorres than was that for the corresponding rates and average spleen for Siniscola and the 3 comparison towns.[2] The level of the average enlarged spleen was similar in the two protected towns at

Table 1—Constants of regression equations fitted to the logarithms of each of four measures of malaria prevalence for the years 1929-1934.

Area	Regression constants: *a* — *Logarithm*	*a* — *Per cent*	*b* — *logarithm*	*b* — *Annual rate of change, per cent*
	Spleen rate			
Portotorres	1.11 ± 0.012	12.8	−0.0235 ± 0.0071	5.26
Siniscola	1.59 ± 0.015	38.9	−0.0267 ± 0.0086	5.96
Lodé, Posada, Torpé	2.00 ± 0.009	100.0	−0.0144 ± 0.0051	3.27
	Parasite rate			
Portotorres	0.634 ± 00.41	4.30	−0.0698 ± 0.0237	14.9
Siniscola	1.08 ± 0.037	11.9	−0.0072 ± 0.0217	1.64
Lodé, Posada, Torpé	1.50 ± 0.050	31.8	−0.0364 ± 0.0295	8.03
	Average spleen			
Portotorres	−0.715 ± 0.012	0.193	−0.0340 ± 0.0069	7.54
Siniscola	−0.304 ± 0.023	0.497	−0.0001 ± 0.0135	0.03
Lodé, Posada, Torpé	0.402 ± 0.012	2.52	−0.0275 ± 0.0072	6.13
	Average enlarged spleen			
Portotorres	0.130 ± 0.003	1.52	−0.0108 ± 0.0015	2.46
Siniscola	0.105 ± 0.014	1.27	+0.0266 ± 0.0080	6.32
Lodé, Posada, Torpé	0.401 ± 0.006	2.52	−0.0134 ± 0.0034	3.03

the beginning of the period. Spleen rates given by the *a* constants for Portotorres, Siniscola, and the comparison towns were 13, 39, and 100 per cent. The parasite rates were 4, 12, and 32 per cent respectively. The average spleens were 0.19, 0.50, and 2.52 specifically. The average spleens in the protected towns given by these constants did not extend to the costal margin even on deep inspiration, while that in the comparison towns extended into the upper half of the area between the costal margin and the umbilicus. The average enlarged spleens in the protected towns lay within the range of spleens palpable on deep inspiration only, while those for the comparison towns extended below the costal margin.

The difference in level between the average spleen and the average enlarged spleen was greatest in Portotorres. In the comparison towns the two measures had the same value. The proportion of children with no spleen enlargement is responsible for these di-

[2] $\text{Standard error}_a = \left\{ \frac{\Sigma \text{ (log observed—log calculated values)}^2}{n(n-2)} \right\}^{\frac{1}{2}}$

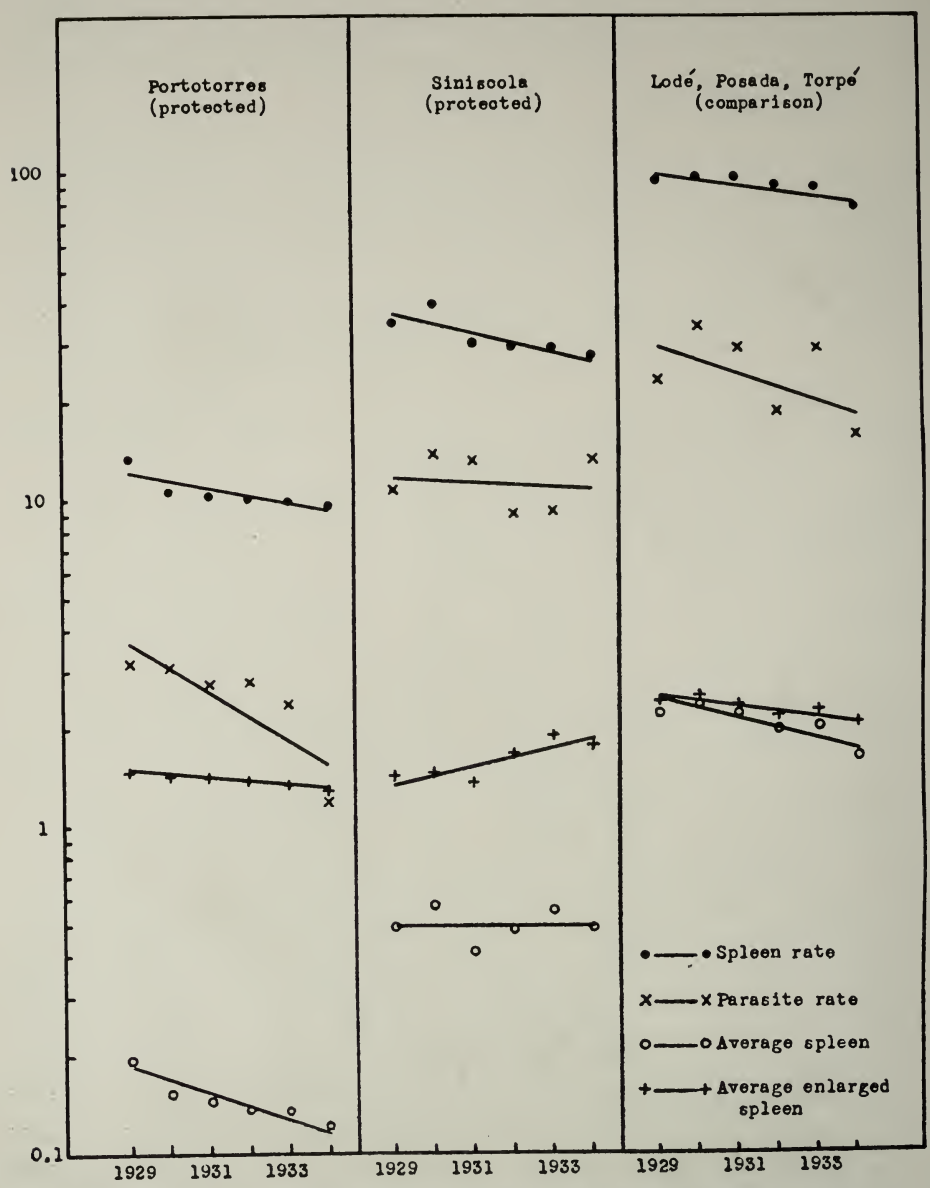

Fig. 8—The level and time trend of malaria indexes in protected and comparison areas for the years 1929—34.

vergences. In the comparison towns, with presumably 100 per cent of the spleens enlarged, the two measures had to be identical.

The *b* constants differentiate the slopes of the lines. Most of these rates of decrease were significant. There is one instance of a significant increase, the average enlarged spleens in Siniscola. Parasite rates dropped more rapidly, but not significantly so, because

of the yearly fluctuations. While it is apparent that all but one of the indexes shown in Figure 8 diminished during the period, the rate of decrease was not great. The lower level of the rates and averages in protected as compared with unprotected areas constitutes the significant difference here.

Table 2—Spleen and parasite rates, by year, in protected and comparison areas.

—	Portotorres (protected)		Siniscola (protected)		Lodé, Posada, Torpé (comparison)	
	Spleen Per cent	*Parasite Per cent*	*Spleen Per cent*	*Parasite Per cent*	*Spleen Per cent*	*Parasite Per cent*
1929	13.3	3.19	34.8	10.7	93.0	22.9
1930	10.7	3.07	39.3	13.7	94.3	33.7
1931	10.3	2.76	30.1	13.0	94.8	28.5
1932	10.1	2.79	29.3	9.01	90.1	18.3
1933	9.85	2.39	29.0	9.11	88.6	28.5
1934	9.61	1.20	27.3	13.1	77.3	15.4
1929-34	10.6	2.55	31.1	11.4	89.4	24.5

The association of malaria parasites with enlarged spleens.

In view of the cross-tabulations made of results of spleen and parasite examinations in 1929-1934 it was possible to examine the data for the presence of association between these two indicators of malaria infection. Table 3 contains the number of individuals recorded for all spleen and blood categories by year for each of the three areas. From the data in columns 2, 3, 10, and 11, fourfold tables were set up like the one below:

Portotorres, 1929

Spleen enlargement	Blood parasites Present	Absent	Total
Present	16	67	83
Absent	4	539	543
Total	20	606	626

Chi square tests were applied to data for each year and for the 6 years combined for each area. P values obtained from Fisher's table for the Chi squares indicate whether negative spleens were associated with negative bloods and positive spleens with positive bloods. One would expect such a relationship, since spleen enlargement and the presence of parasites are both presumably evidence of infection.

Results of the Chi square tests applied to the data in fourfold tables set up for Portotorres by year and for all years combined indicate that a significant association did exist between these two factors, in spite of the fact that there were always children with para-

sites whose spleens were normal (4 in the table for Portotorres, 1929, above) and children with enlarged spleens but no parasites (67). The value of P in every test was less than 0.01, which signified that the chances were less than one in 100 that the individuals recorded were randomly distributed with respect to these four categories.

Similarly significant association was found between results of spleen and blood examinations in Siniscola for all years combined and for each year separately except 1932. Data for the comparison

Table 3—The results of malaria surveys, by year, in protected and comparison areas among children from 1 to 12 years of age.

Year	Month	Bl O Sp O	Bl O Sp +	*Vivax* + Sp O	*Vivax* + Sp +	*Faliciparum* + Sp O	*Faliciparum* + Sp +	*Malariae* + Sp O	*Malariae* + Sp +	*Bl* + Sp O	*Bl* + Sp +	total exmd
(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
						Portotorres (protected)						
1929	Jan.	539	67	2	9	1	7	1	0	4	16	626
1930	Mar.	513	56	10	5	1	2	0	0	11	7	587
1931	Mar.	573	60	10	4	1	1	0	2	11	7	651
1932	Mar.	568	60	11	5	2	0	0	0	13	5	646
1933	Mar.	597	57	5	6	2	3	0	0	7	9	670
1934	Mar.	598	60	4	2	0	2	0	0	4	4	666
1929-1934		3388	360	42	31	7	15	1	2	50	48	3846
						Siniscola (protected)						
1929	Jan.	152	66	3	8	4	11	0	0	7	19	244
1930	Mar.	196	94	6	32	2	6	0	0	8	38	336
1931	Mar.	265	96	17	20	7	8	1	1	25	29	415
1932	Mar.	291	113	14	12	9	3	0	2	23	17	444
1933	Mar.	276	103	11	12	9	5	0	1	20	18	417
1934	Mar.	308	71	8	26	1	19	0	3	9	48	436
1929-1934		1488	543	59	110	32	52	1	7	92	169	2292
						Lodé, Posada, Torpé (comparison)						
1929	April	15	160	1	21	0	30	0	0	1	51	227
1930	Mar.	26	334	3	137	2	35	0	6	5	178	543
1931		28	388	1	83	1	70	0	11	2	164	582
1932		67	528	3	87	2	38	0	3	5	128	728
1933		67	371	2	100	1	68	0	4	3	172	613
1934	Jan.	116	346	5	50	3	24	0	2	8	76	546
1929-1934		319	2127	15	478	9	265	0	26	24	769	3239

towns failed to show significant association in 1929 because of the very small number (16) of children who had normal spleens. Data for 1930, however, gave a P value of 0.03, which is considered within the range of probable significance and those for subsequent years, and for all years gave P values of less than 0.01. In spite of the large number of children in these comparison towns with enlarged spleens and no parasites, there was a significant tendency for those with normal spleens to be found in the no parasite group.

The average spleen associated with specific parasites.

The question has been frequently raised of whether the degree of spleen enlargement among persons with falciparum infections is

greater or less than among persons with vivax or malariae infections. In the present analysis the question with respect to falciparum and vivax infections has been investigated, but the number of persons with malariae parasites was too small to permit evaluation of their spleen enlargement. Table 4 contains the spleen averages by year and area for children with diagnosed falciparum or vivax infections, for children with any parasites, for those with no parasites, and for all children. The average enlarged spleens are also given in this table. Variance analysis was applied to these means. In Portotorres alone was the average falciparum spleen for the period as a whole (1.41) significantly larger than the average vivax spleen (0.685). The P value was less than 0.01. In each area children with parasites had larger average spleens than those with no parasites ($P<0.01$), which was to be expected since children with parasites were all infected.

The meaning of an average spleen pertaining to falciparum in-

Table 4—The average spleen and average enlarged spleen, by year, with respect to parasite present in protected and comparison areas.

Population Group		1929	1930	1931	1935	1933	1934	1929-1934
Portorres (protected)								
Average spleen								
Parasite	Falciparum	2.00	1.33	0.500	——	1.40	1.50	1.41
	Vivax	1.18	0.600	0.429	0.500	1.00	0.500	0.685
	Malariae	——	——	2.00	——	——	——	1.33
	All parasites	1.45	0.722	0.688	0.444	1.12	0.750	0.867
	No parasites	0.154	0.135	0.133	0.131	0.110	0.116	0.129
	Total	0.195	0.153	0.146	0.139	0.134	0.123	0.148
Average enlarged spleen		1.47	1.43	1.42	1.38	1.36	1.28	1.39
Siniscola (protected)								
Average spleen								
Parasite	Falciparum	1.20	0.875	1.07	0.583	0.643	1.75	1.10
	Vivax	1.09	1.45	0.865	0.846	0.957	1.38	1.12
	Malariae	——	——	1.00	2.00	2.00	2.67	2.00
	All parasites	1.15	1.35	0.926	0.825	0.868	1.58	1.14
	No parasites	0.413	0.452	0.332	0.450	0.515	0.325	0.414
	Total	0.492	0.574	0.410	0.484	0.547	0.489	0.497
Average enlarged spleen		1.41	1.46	1.36	1.65	1.88	1.79	1.76
Lodé, Posada, Torpé (comparison)								
Average spleen								
Parasite	Falciparum	2.70	2.86	2.46	2.32	2.29	1.78	2.41
	Vivax	2.36	2.74	2.65	2.30	2.24	2.04	2.44
	Malariae	——	3.00	2.36	2.33	3.25	2.50	2.65
	All parasites	2.56	2.77	2.55	2.32	2.28	1.96	2.44
	No parasites	2.10	2.09	2.05	1.86	1.84	1.53	1.88
	Total	2.20	2.32	2.19	1.95	1.96	1.60	2.02
Average enlarged spleen		2.37	2.46	2.31	2.16	2.22	2.06	2.25

fected persons as compared with one of those infected with vivax or malariae, as defined by the data given, calls for comment. Although it was possible in the laboratory to ascribe an infection to one rather than another of the malaria plasmodia, experience has indicated that in highly endemic areas children are likely to be infected with all three at an early age. The laboratory diagnosis is determin-

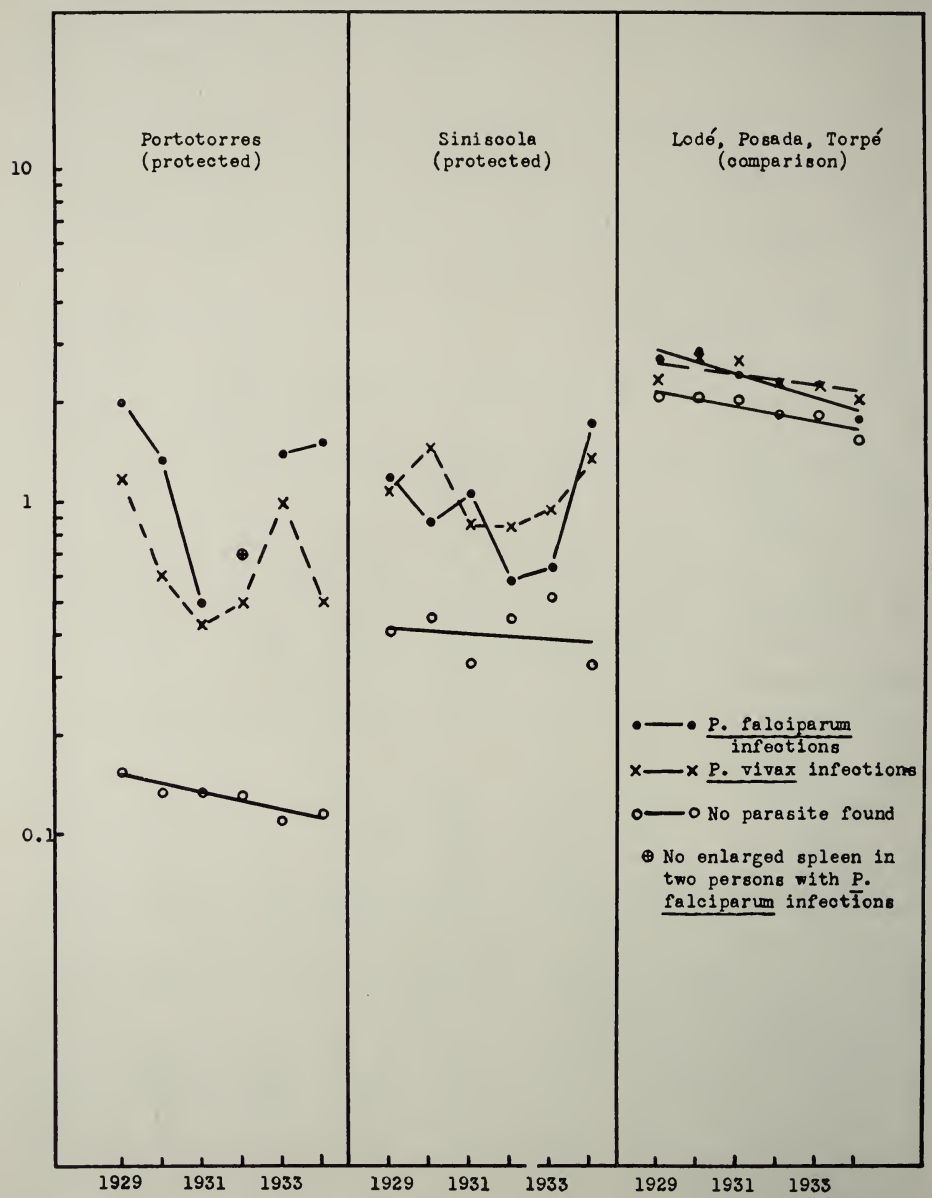

Fig 9—Time changes in average size of spleen in children in protected and comparison areas for the years 1929-34.

ed, therefore, by the parasites of the particular plasmodium predominating in the peripheral blood at the time the smear is made. Consequently, spleen averages ascribed to one rather than another species in this analysis may have little meaning. How significant is the greater enlargement shown by children in Portotorres having falciparum infections is another matter, however. It is possible that during the long period of antilarval activity in this area the multiple infections may have decreased to a point where an actual species difference may have emerged.

The changes with time of the average spleens of children with falciparum and with vivax infections and of those with no parasites are shown in Figure 9. In Portotorres and in Siniscola the number of children with parasites in these years was small and the averages fluctuated greatly. In Portotorres the five falciparum averages shown were higher than the vivax averages. This was not true of Siniscola. In the comparison towns the average for the two species of infecting parasites overlapped and the course of each series was rectilinear. The average spleens for children with no parasites lay at varying distances below those for children with parasites. These differences in level were due to the proportion of truly uninfected children included.

Straight lines have been fitted to the logarithms of the series of average spleens which could be appropriately so treated. The annual rates of decrease (*b* constants) for the fitted lines are:

Regression coefficient (*b* constant)

	Logarithm with standard error	Annual rate of decrease Per cent
Portotorres: No parasites	− 0.025 ± 0.006	5.68
Siniscola: No parasites	− 0.006 ± 0.021	1.42
Comparison towns:		
Falciparum	− 0.035 ± 0.008	7.71
Vivax	− 0.018 ± 0.009	4.12
No parasites	− 0.024 ± 0.007	5.30

These were statistically significant except the rate for Siniscola for children showing no parasites. Figure 9 indicates that the slope in this instance was negligible. The rates of decrease for Portotorres and the comparison towns for average spleens among children with no parasites were identical. Falciparum spleens in the comparison towns decreased more rapidly than vivax spleens but the difference was not significant. These averages apply to known infected individuals and were observed in towns in which treatment was the only antimalaria measure employed. It is of interest, therefore, to find a significant decrease occurring.

Prevalence of parasite species and their ratios in the comparison area.

In view of the amount of malaria present in the comparison towns it was believed that the investigation of the prevalence of each species and of their ratios should be limited to data collected in these areas. Prevalence rates are shown in Table 5 by year and for the period as a whole. Vivax infections were more numerous than falciparum infections in every year except 1929. In 1930 the vivax rate was 26 per cent as compared with a falciparum rate of 7 per cent. So far no mention has been made of malariae infections, for the reason that only 26 children were found to have these infections in the course of the 6-year interval (Table 3).

Table 5--Parasite prevalence rates in comparison towns, by year.

Year	Falciparum Per cent	Vivax Per cent	Malariae Per cent	Total Per cent
1929	13.2	9.7	—	22.9
1930	6.8	25.8	1.1	33.7
1931	12.2	14.4	1.9	28.5
1932	5.6	12.4	0.3	18.3
1933	11.3	16.6	0.7	28.5
1934	4.9	10.1	0.4	15.4
1929-1934	8.5	15.2	0.8	24.5

Parasite ratios, based on data for all years for children with enlarged spleens and for those with normal spleens, were almost identical: approximately 35 per cent for children with falciparum infections and 62 per cent for those with vivax infections. The spleens of the 26 children with malariae infections were all enlarged (3 per cent). Parasite ratios by year are shown in Table 6, and a Chi square applied to the data from which they were derived gave a P value of less than 0.01. This means that the variations found probably reflect a real difference in incidence of the three parasites in different years. Malariae infections were too few for serious consideration. Falciparum infections dropped from 58 per cent in 1929 to 20 per cent the following year. Vivax infections rose correspondingly. The ratios fluctuated from year to year, but there was no tendency for those of one parasite to increase at the expense of those of another as the period advanced.

Table 6—Parasite ratios in comparison towns, by year.

Parasite	1929 Per cent	1930 Per cent	1931 Per cent	1932 Per cent	1933 Per cent	1934 Per cent	Total Per cent
Falciparum	57.7	20.2	42.8	30.8	39.4	32.1	34.7
Vivax	42.3	76.5	50.6	67.7	58.3	65.5	62.2
Malariae	—	3.3	6.6	1.5	2.3	2.4	3.2
TOTAL	100.0	100.00	100.0	100.0	100.0	100.0	100.1

Discussion

That the amount of malaria in Portotorres was greatly reduced during the period of antilarval operations has been shown by the analysis of time trends in Figure 7. One factor which complicated the malaria situation in both Portotorres and Siniscola was the annual migration of agricultural workers from the towns in the summer. These people brought malaria back with them and, with anophelines present, transmission could and did occur. Portotorres was, however, not considered a malarious community. This is shown by the fact that in 1927 a hotel and restaurant were opened and a bathhouse built. People came to town for the summer to escape malaria elsewhere. Families began to move in. Many of these people also brought malaria with them. It is gratifying, therefore, to find that the disease was reduced to a negligible amount in spite of the incomplete control of anophelines achieved.

Malaria decreased during the years 1929-1934 in comparison as well as in protected areas. The intensive treatment programs in Posada and Torpé were partly responsible for this, but not entirely so since spleen and parasite rates also decreased in Lodé, where only routine measures were employed.

The fact that spleen rates were always three or more times greater than the parasite rates in these communities (Table 2) deserves comment. This relationship has been observed in many malarious areas bordering the Mediterranean in surveys made in the winter when residual malaria was at a minimum. Only during the summer epidemic did the parasite rates equal or exceed the spleen rates. It is for this reason that the spleen rates and average spleens furnish the best measure of malaria prevalence in these areas.

We have not been able to demonstrate a significant difference in the amount of spleen enlargement produced by parasites of falciparum malaria as compared with those of vivax malaria, except in the averages for the period (1929-1934) for Portotorres. The reason for this is probably that many of the infections were initially mixed and that the particular parasite identified was dependent upon which one was circulating in greater numbers at the time the blood smear was made. The long period of antilarval measures in Portotorres, however, may have reduced the number of mixed infections, so that the true effect of a given parasite on the spleen became apparent.

For an adequate appraisal of a field project, data from a comparison area are needed.The reasons for this are obvious. The height of the malaria endemic fluctuates from year to year. There are cycles within the endemic extending over several years. It is diffi-

cult to evaluate antilarval or any other preventive measures applied in one area if we do not follow concurrently events in an area where these measures are not in operation. In Sardinia, observations were begun in 1925 in Portotorres, in 1927 in Siniscola, and in 1927 and 1928 in the comparison towns. While the endemics in Portotorres and Siniscola may have been comparable, we cannot be sure because we do not have data for both areas over the entire period. It seems probable that the endemic was more intense in the three comparison areas than in either of the protected towns. The initial spleen rate in Portotorres was 47 per cent; the rates for the comparison towns when first surveyed were: Lodé 87 per cent, Posada 100 per cent, and Torpé 75 per cent. It is difficult to set up anything like a laboratory controlled experiment in the field when working with human populations, but failure to do so makes it difficult to appraise the results of a project satisfactorily.

An effort has been made in the analysis of the data to use only simple and apt statistical techniques. The analytical tools are never an end in themselves. They merely enable one to present the data in such a way that the salient features may be easily grasped. If a project is planned and operated so that the data collected give measures that are comparable, not only from year to year but from area to area, little analysis is required. Since the production of both anophelines and plasmodia is multiplicative, time changes should be placed on a logarithmic scale both in plotting and in fitting regression lines. Variance analysis may be used to test the significance of averages and Chi square tests to determine the presence of association.

Summary and Conclusions

A malaria control project based on antilarval measures was conducted in Portotorres, Sardinia, during the years 1925-1934 by the Malaria Experiment Station in Italy. Similar measures were in operation in the town of Siniscola in 1928-1934. Three other Sardinian towns were observed as comparison areas. Intensive treatment campaigns were conducted in two of these comparison towns and routine treatment only in the third.

The object of this study has been (1) to describe the course of the malaria endemic in these towns and (2) to apply analytical methods best suited to bring out time trends, area differences, and interrelationships of the indexes employed. These indexes were: anopheles density, malaria case and infant infection rates, spleen and parasite rates, average spleen and average enlarged spleen derived from data collcted in winter surveys of children from 1 to 12 years of age.

The results were as follows:

1. Anopheles density and malaria prevalence in the protected town of Portotorres declined sharply in the years 1925-1929 to a status of comparative equilibrium at a low level. Spleen and parasite rates as well as average spleens were significantly lower in Portotorres than they were in the other areas during the years 1929-1934.

2. The malaria endemic in the comparison areas also declined significantly in the years 1929-1934, but its level was well above that in either of the protected towns of Portotorres or Siniscola.

3. An interval of seven weeks was observed between the summer rise in anopheles density and the corresponding rise in malaria cases. This suggests that two vivax transmission cycles were needed to produce the seasonal epidemic.

4. The average spleen of children with parasites was significantly higher in all areas than that of children with no parasites.

5. Children in Portotorres showing parasites of falciparum malaria had a larger average spleen than children with vivax malaria. This was not true of children in other towns, however.

6. A logarithmic scale is best suited to bring out similarities in the time changes of the indexes. Regression lines have been fitted to logarithms of the measures so that annual rates of decrease might be determined. The multiplicative character of the basic data requires this treatment. Other statistical tests may be employed to determine significance of means or the presence of association.

7. A project planned to include a protected and a comparison area and the collection of comparable data over a period of years should enable the malariologist to evaluate the results with a minimum of statistical treatment.

References

Fisher, R. A.
1944 Statistical Methods for Research Workers. 9th Edition. Oliver and Boyd, London.

Hackett, L. W.
1929 Malaria control through anti-mosquito measures in Italy. Trans. Roy. Soc. Trop. Med. and Hyg., 22: 477-499.
1944 Spleen measurement in malaria. J. Nat. Malaria Soc., 3:122-133.

Howard, H. H., W. C. Earle, and H. Meunch
1935. A method of analysis of field malaria data. J. Am. Statist. Assn., 30: 249-256.

Kitchen, S. F., and Persis Putnam.
1942 Observations on the mechanism of the parasite cycle in *falciparum* malaria. Am. J. Trop. Med., 22: 361-386.

Snedecor, George W.
1934 Calculation and interpretation of analysis of variance and covariance. Collegiate Press, Inc., Ames, Iowa.

MALARIA RESEARCH AT THE UNIVERSITY OF TENNESSEE COLLEGE OF MEDICINE, MEMPHIS

Clinical testing of drugs for prophylactic and suppressive activities has been the principal line of investigation since 1943. The studies have been carried on under an O. S. R. D. contract. About 400 neurosyphilitics given malaria therapy have constituted the patients in whom the drugs were studied. At present the McCoy strain of *Plasmodium vivax* and the Costa strain of *P. falciparum* are maintained; both are available as sporozoites.

There are now 20 beds at Gailor Hospital available for neurosyphilitics, and it is expected that completion of two unfinished wings will materially increase the capacity. A federal grant-in-aid has been obtained to support the work when the O.S.R.D. contract expires at the end of June.

During the past year there have developed several studies on the pre-erythrocytic stages of plasmodia. These investigations are being pursued by implanting mosquito salivary glands infected with sporozoites onto the chorio-allantoic memberanes of chick embryos, onto tissue grafts previously made on such membranes, and into tissue cultures made according to the usual techniques. Both avian and human plasmodia are being used for these studies.

Preliminary studies on pathological physiology in the malarious human have stimulated the development of more intensive research along these lines in monkeys infected with *P. knowlesi.* Complete determinations of blood volume, blood specific gravity, arteriovenous oxygen differences, extra-cellular fluid volumes, etc., are being made in monkeys with fatal infections and in non-infected controls, as well as in infected monkeys under various therapeutic regimes.

Simulation of certain effects produced by malaria parasites has been attempted by using carbon particles of various sizes injected into the blood streams of the commoner laboratory animals. It. is hoped that this method of study will permit some observations which might be difficult to carry on in the few species of laboratory animals which are susceptible to plasmodia.

Earlier studies on the complement fixation reactions in malaria have led to attempts to determine whether soluble antigens, detectable by this type of test, can be demonstrated in the blood or various fixed tissues of different animals infected with several species of plasmodia.

The Tiselius apparatus has been employed from time to time in conjunction with the various studies on human and animal malarias. —*From the Committee on Medical Research.*

VERTICAL DRAINAGE FOR MOSQUITO CONTROL ON A SOLOMON ISLAND BASE

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(Received for publication 26 October 1945)

An unusual feature of the topography of this island is the large number of small sink hole depressions, usually less than a hundred yards square and from four to ten feet deep, which are abundant throughout the level areas. The recent origin of these depressions in the hard clay topsoil is suggested by the presence in them of cocoanut trees, many of which are now growing in several feet of water. It is quite possible that earthquakes, which are of frequent occurence in this area, or the leaching away of a portion of the underlying foundation of coral are the processes provoking the formation of these sink holes.

These depressions are relatively poor producers of anophelines, but neverthless demand constant attention and therefore justify a reasonable effort to eliminate them as mosquito breeders. Top-feeding minnows thrive in the ponds but are efficient in mosquito control only when the edeges are kept free of vegetation and the pond surface kept free of sprouting cocoanuts. This cleaning, in itself, requires considerable labor and therefore vertical drainage through the nonporous topsoil into the porous underlying coral was resorted to.

The simplest method that has been used is the blasting of a hole through the topsoil in the bottom of the sink hole with dynamite. Flow usually continues in this case for only a short time, however, before silt clogs the drain. Another and more successful method has been to dig a hole down to the coral at the edge of a sink hole with a dragline. This type of drain usually remains open long enough to drain the pond, but not long enough to justify the expense of the use of heavy equipment.

Experiments were then begun with the end in view of providing a filter that would prevent clogging of the drain opening into the coral substrate. At first a well rig was used to drill an eight inch hole thirty feet into the ground, approximately fifteen feet of it being into coral. An excavation five feet by five feet and five feet deep was made around the eight inch hole, and lined with a wooden frame. An eight inch pipe thirty feet long was placed inside the drilled hole. This opening into coral was connected to the sink hole by a ditch one foot wide and about ten feet long. It was

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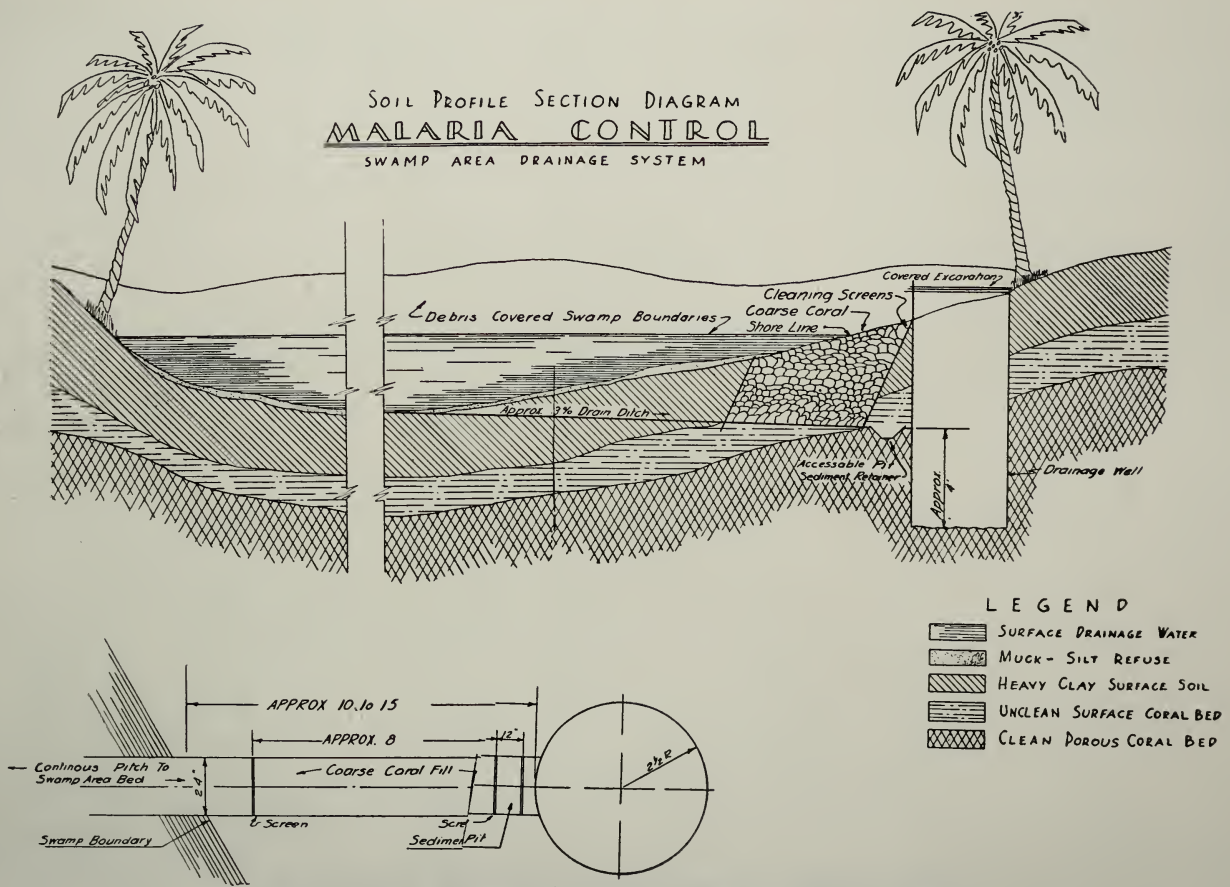

Fig. 1—Section Diagram of Verticle Drainage for Malaria Control

screened on both ends to keep out large pieces of debris such as cocoanuts, and the ditch was filled with coral to act as a filter to the soil in the water. Thus fairly clean water hit the vertical drainage hole. This type of drainage worked fairly well in two cases, but the flow off was not fast enough due to the fact that insufficient coral surface was exposed to absorption. Also, if the opening stopped up, the well rig would have to be set up to clean it out.

Therefore, a simpler plan was tried and it has proven very satisfactory. The well rig was dispensed with and a six-foot square hole dug down to coral. The hole was then deepened two or three feet more. This excavation has usually been placed ten to twelve feet from the sink hole to be drained. A ditch one foot wide and as deep as is necessary to drain the pool is then dug to connect the two. A screen is then placed at each end of the ditch and it is filled with coral. The six by six foot square hole drains the water off at a rapid rate. Ponds 100 feet square and eight feet deep have been drained in a day. If the silt gets by the coral filter and stops up the drainage surface, it can easily be cleaned out by taking the cover off the hole and shoveling out the silt. This work is being done by native labor with picks and shovels and the expense is practically zero. Utilizing this simple vertical drainage system as shown in figure 1, it has been possible to drain a considerable number of the sink holes on this island. These depressions have remained dry for ten months.

CORRECTION

The article titled "Differentiating the Larvae of *Anopheles georgianus* King, *A. bradleyi* King, and *A. punctipennis* (Say)", by Captain Virgil I. Miles, which appeared in the September, 1945, issue of the *Journal*, contained the following errors:

Page 236, line 14 of the table text (in small print), the first word "on" changed to "or".

Page 238, lines 11 to 14 of the table text (in small print), should be deleted and the following lines substituted:

Spicules of antennae	fine, mostly slender and not pigmented	coarse, spinelike and pigmented

Page 239, line 22, the figure "5" should be inserted at the end of the line, to read "usually with 3 to 5."

REPORT OF EDITORIAL BOARD, 1945

The Editorial Board met in executive session in Atlanta, Georgia, on January 12, 1945. The meeting was attended by Dr. Mark F. Boyd, both as an *ex officio* member and as Secretary-Treasurer of the Society; and also by the publisher of the Journal, Mr. H. R. Archer of Archer and Smith Printing Company, Knoxville, Tennessee. Decisions on editorial policy taken by this meeting are largely incorporated in a statement which has appeared under the title "Material Submitted for Publication" on the inside of the front cover of the last two issues of the Journal.

The Editorial Board has endeavored to improve the appearance and scientific quality of the Journal by means of changes in format, which were adopted with the current volume, and by careful review of articles. Papers received by the office of the editor are referred to one or more editorial consultants who are particularly qualified to give an opinion on the subject treated. This policy has resulted in a few refusals to publish articles submitted, but it is believed that its continuation will make the journal increasingly attractive as a medium of publication and will result in the submission of a larger number of articles for publication. As a matter of fact, there is already evidence to this effect: All articles derived from the last annual meeting were published in the current issue of the Journal or in prior issues, but more than enough articles are in hand for the December issue.

An effort has been made to make each issue of the Journal interesting to the entire membership of the Society which is, of course, composed of representatives from a fairly wide variety of professional fields; also to make each issue as timely as possible. It will be noted that all articles in the current volume bear a notation of the date the article was received for publication. This has been done for obvious reasons and for the additional reason that it is not always possible to publish an article expeditiously. Each issue has a limited number of pages and its makeup requires a careful selection of papers of proper length in addition to other considerations. For this reason, authors may occasionally correct galley proof in the expectation that their article is to appear in the next issue, only to find that it is not published until the next succeeding issue.

The current volume of the Journal has been limited to approximately 400 pages, of which 278 have been published through the third number. The material published has fallen roughly into the following categories:

Articles on	Number	Pages	Per cent of total pages
Transactions or Business of Society	8	14	5.0
Medical Malariology	10	77	27.2
Entomology	10	61	21.9
Malaria Control Engineering and operations	12	116	41.7
Miscellaneous	2	10	4.2
Total	42	278	100.0

It would appear from this classification that the material published in the current volume is rather heavily weighted by articles on malaria control engineering. As a matter of fact, the classification is obviously a very broad one in all categories, and actually there were only two purely engineering articles published, having a total of twelve pages or a little more than four per cent of the total number of pages published. The Editorial Board believes that a larger number of technical papers on engineering should be published, and solicits the cooperation of the Society in this regard.

Also solicited are brief notes of research or of matters calculated to be of interest to readers of the Journal, such as "Letters to the Editor" which appear in other journals.

With regard to editorial policies, the Board wishes to solicit comments by the members of the Society in general and with special reference to the policy governing the acceptance by the Journal of papers read before the annual meetings of the Society. Some criticism has been received to the effect that in as much as the Editorial Board reserves the right to refuse to publish papers read at the annual meeting, authors should have a similar right to withdraw such papers from publication by the Journal.

The Editorial Board is aware of the fact that numerous mistakes have been made in printing articles and that there has been delay in the appearance of issues of the Journal and in the receipt of reprint orders by authors. To a large extent perhaps these difficulties have been due to the war, but if they do not soon disappear, the Editorial Board wishes to recommend that other arrangements be made for the publication of the Journal.

The current Editor of the Journal has submitted his resignation effective January 1, 1946, because the nature of his work in 1946 will make it physically impossible for him to continue in his present capacity. Another member of the Editorial Board, Doctor Robert L. Usinger, has asked to be relieved of duty on the Editorial Board as soon as his place can be taken by Doctor Lloyd Rozeboom, for whom he has been substituting for the past year. The Editorial Board urges that careful consideration be given to the successor of the current editor with particular reference to the selection of a per-

son with editorial experience. It is the belief of the Board that the Editor should be selected primarily because of his editorial qualifications, but that, if possible, he should be also a medical malariologist.

On the basis of experience during the current year, the work of the office of the editor of the Journal requires considerable time in addition to the regular duties performed by a secretary. Therefore, the Board wishes to recommend that modest financial arrangements be made to supply the editor of the Journal with secretarial assistance and suggests that this might be done in the form of an honorarium.

Respectfully submitted,
Robert Briggs Watson, M. D.
Chairman and Editor of Journal
Robert L. Usinger
Louva G. Lenert
Mark F. Boyd, M. D., *ex officio*

REQUEST FOR BACK NUMBERS

The supply of early issues of the "Journal of the National Malaria Society" is exhausted. The Office of the Secretary needs several numbers to complete its files. Also, requests are being received from libraries and others for back numbers. Individual subscribers who have or know of copies which can be spared would do a distinct service to the Society by making available such copies to the Society. Communications in this regard should be addressed to the Secretary who will buy, at his discretion and indicated prices, copies of the following back issues of the Journal:

Volume I—$2.00 Volume II, No. 1—$1.00

Volume III, Nos. 1-2—50¢ (each)

PRECIPITIN TEST FOR DETERMINING NATURAL INSECT PREDATORS OF IMMATURE MOSQUITOES

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(Received for publication 25 June 1945)

It has been demonstrated that the surface feeding minnow, *Gambusia,* is capable of destroying great numbers of immature mosquitoes (Hildebrand, 1925), and that under certain conditions the introduction of these small fish constitutes a good naturalistic method of controlling malaria (Russell and Jacob, 1939). Evidence of the feeding habits of *Gambusia* can be obtained by observing the engulfing of mosquitoes in the laboratory and also by examining the stomach contents of specimens caught in nature for the chitinous bodies of ingested larvae and pupae (Hess and Tarzwell, 1942).

There are many other organisms that may likewise act as efficient predators, but very little is known about their feeding habits, particularly in their natural environment. Although various aquatic insects have been observed to ingest numerous mosquito larvae in the laboratory, it is considered unwise to conclude that a certain insect is a good natural predator on the basis of such observations (Hinman, 1934). It is essential to determine if the insect under natural conditions selects mosquitoes even though a wide variety of other foods are available. Unfortunately, since many predatory insects only suck out the fleshy internal structures of their prey and discard the chitinous exoskeleton, it is not possible, as with *Gambusia,* to determine their natural food selections by examining microscopically their stomach contents.

The following experiments were performed to determine if a precipitin test might determine the presence of anopheline mosquitoes ingested by aquatic insect predators. The principle involved is the same as that used by Bull and King (1923) to identify the source of the blood meals of the adult mosquitoes. In the present study, antibodies were produced in the serum of a rabbit which were capable of forming a precipitate when brought in contact with extracts of anopheline pupae, the homologous antigen. A series of precipitin tests was performed in which this antiserum was overlayed with extracts of various mosquitoes and of insect predators that had fed upon immature mosquitoes or had been starved. The formation of precipitates at the interphases of the two liquids was

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considered as indicating the presence of anopheline substance or substances antigenically similar to the homologous antigen.

Materials and Methods

Production of an Anopheline Pupal Antiserum. The antiserum for the precipitin tests was obtained from a rabbit after the intraperitoneal inoculation of anopheline pupae. Inasmuch as pupae do not feed, it was considered advisable to use them instead of larvae which might contain antigenic proteins of engulfed bacteria, protozoa, and so forth. Live pupae of *A. quadrimaculatus* raised in the Wilson Dam insectary were washed by repeatedly suspending them in copious amounts of sterile water and by centrifugation. The washed pupae were macerated in sterile physiological salt solution in a proportion of 100 pupae to one cc. of saline solution. The resulting heavy suspension of pupal material was centrifuged lightly to remove coarse particles. A rabbit was inoculated on alternate days with increasing amounts of this antigenic suspension. Six inoculations were made with material from the following number of pupae: 50, 100, 150, 390, 398, and 418. Nine days after the final inoculation the rabbit was exsanguinated and the serum collected. It was kept frozen in a refrigerator except when tests were being performed.

Concentration of Homologous Test Antigen. In order to discover the titer strength of the antiserum, it was necessary to determine the maximum concentration of the homologous antigenic substance. Forty pupae of *A. quadrimaculatus* were washed in several changes of water and pipetted onto a paper towel. They were then transferred by means of a camel's hair brush to a dry watch glass. The watch glass with the pupae was placed for one hour in a dessicator containing "Drierite". It is believed that this drying process removed only the external moisture from the pupae for at the end of the drying period many of the pupae still showed signs of life. The forty pupae weighed 0.0968 gram.

The plunger was removed from a 2 cc. syringe and the needle end was plugged. The short neck of the syringe was filled with a drop of water. The forty "dried" pupae were then introduced and thoroughly macerated with a glass rod. One cubic centimeter of water was added to the pupal substance. The graduations on the syringe indicated that the forty pupae occupied 0.156 cc. On the basis of this figure, it would require 256 pupae to provide 1 cc. of pupal substance.

Performing of Test. All of the precipitin tests were performed in practically the same manner. The test antigens were prepared by first washing the organism (mosquitoes and insect

predators) in several changes of water. Then the organisms were macerated in a given quantity of physiological salt solution (usually 1.25 cc.) and incubated at 37°C. for 1 to 2 hours. For convenience, on several occasions the suspensions were refrigerated at 6° C. for 20 to 24 hours. The extracts of the organisms were filtered through asbestos fibers dried in the bottom of small perforated porcelain cups. The resulting filtrates were usually crystal clear and free from any coloration. They were used directly as the test antigens or were added to given quantities of saline solution in order to prepare higher dilutions. The antiserum was always used diluted with one part of physiological saline (1:2 dilution).

In setting up the tests, the antiserum was introduced into the test tubes first. Then in order to obtain a ring reaction, the antigen was carefully floated on top of the antiserum by employing a 2-inch 20-gauge needle attached to a one cc. syringe. The tests were performed in test tubes 10 x 75 mm. or 5 x 50 mm. size. When the larger tubes were used, 0.2 cc. of both the antiserum and antigen were introduced into each tube. Only one half this quantity was used with the smaller tubes.

After filling, the tubes were allowed to stand undisturbed at room temperature (between 85° and 95°F.) or placed in an incubator at 37° C. At the end of one or two hours the reactions were graded according to the intensity of the rings at the interphase between the two liquids. Usually when the larger tubes were used it was not possible to float the antigens on top of the antiserum. In that case, all of the tubes were shaken thoroughly, and the test read by the quantity of precipitate present after centrifugation for 5 minutes at 2,000 r.p.m.

Titration of the Anopheline Pupal Antiserum Against the Homologous Antigen. The titer of the antiserum was determined against gradually increasing dilutions of the homologous antigen (pupae of *A. quadrimaculatus*.) In two such titrations the diluted antiserum (1:2) gave a positive reaction with antigen dilutions above 1:4320. Several weeks later after all of the experimental tests had been performed, the titer of the antiserum was found to be between 1:480 and 1:960. This decrease was to be expected since it is known that the titer of most antisera diminish gradually upon storage except when dehydrated from the frozen state (Flosdorf and Mudd, 1938). In performing these titrations the following controls were included: antiserum against normal saline solutions, and antigen dilutions against normal saline solution and normal rabbit serum (diluted 1 in 2 with saline). Reactions only occurred between the antiserum and antigen.

Precipitin Tests With Anopheline Pupal Antiserum

A. Against Anopheline and Culicine Mosquitoes

After determining that the antiserum would react with pupae of *A. quadrimaculatus,* the homologous antigen, it was desired to know if it would form precipitates with other stages of the same species and with the various stages of culicine mosquitoes.

Test 1. In order to prepare approximately equal concentrations of the test antigens, rough estimates were made of the relative sizes of the various stages of the mosquitoes available. The pupae of *A. quadrimaculatus* appeared to be twice the size of the other other specimens. Therefore, the following number of organisms were extracted in 2 cc. quantities of saline solution: 8 pupae of *A. quadrimaculatus,* 16 larvae of *A. quadrimaculatus;* 16 pupae of *C. restuans* and 16 larvae of *C. restuans.* This number of anopheline pupae constituted an original concentration of 1:65 for the homologous antigen. The higher dilutions of all the test antigens were made with this figure representing the original concentration.

The protocol was set up in the larger test tubes. After being thoroughly shaken, the tubes were incubated for one hour and refrigerated. Two hours later, the tubes were centrifuged for 5 minutes and examined for the presence of precipitates (Table 1).

Table 1—Precipitin Test with Anopheline Pupal Antiserum Against Immature Stages of Anopheline (*A. quadrimaculatus*) and Culicine (*C. restuans*) Mosquitoes

Tube Number	Dilution of Antigen	Precipitates after one hour incubation and 5 minutes centrifugation (2000 r.p.m.)			
		Anopheline		Culicine	
		Pupae	Larvae	Pupae	Larvae
1	1:65	++++	++	++++	++
2	1:130	++++	+	+++	+
3	1:320	+++	±	+±	+
4	1:640	++	0	+	±
5	1:1600	+	0	±	0
6	1:2400	±	0	0	0
7	1:3200	0	0	0	0
8	1:4800	0	0	0	0
	Controls against saline solution				
9	Antiserum	0	(broken)	0	0
10	Antigen (1:1600)	0	0	0	0

Test 2. The 1:130 dilutions for the four mosquito extracts prepared for the above tests were used in setting up ring precipitin tests. Included in the protocol were four other test antigens which were prepared from adults of *A. quadrimaculatus,* adults of *C. restuans,* and larvae and pupae of *C. fatigans.* The approximate dilution of these extracts was 1:320. The antigenic substances were

carefully floated on top of equal quantities of antiserum placed in the bottom of the smaller test tubes. (This procedure involving the smaller tubes was used in all of the subsequent tests.) The tubes were kept at room temperature for two hours and examined for the presence and intensity of rings (table 2).

Results. In test 1, a distinct precipitate was formed in the tube containing the antiserum and the 1:1600 dilution of the anopheline pupal extract (homologous antigen). A doubtful positive was obtained with the 1:2400 dilution. Precipitates were also formed with somewhat lower dilutions of the extracts of anopheline larvae, culicine larvae and culicine pupae. Similar results were obtained in test 2; but, in addition, reactions occurred between the antiserum and the extracts of anopheline and culicine adults. The strongest reactions occurred with the pupal extracts which might indicate that the serum was more specific for that particular stage than for a given species of mosquito.

Table 2—Precipitin Test with Anopheline Pupal Antiserum Against Various Stages of Three Mosquitoes (*A. quadrimaculatus, C. restuans,* and *C. fatigans*)

Tube Number	Mosquito Antigens (with estimated dilution)			Intensity of Rings after 2 hours
1	*A. quadrimaculatus*	larvae	(1:130±)	++
2	" "	pupa	(1:130)	++++
3	" "	adult	(1:320±)	++
4	*C. restuans*	larva	(1:130±)	+
5	" "	pupa	(1:130±)	++
6	" "	adult	(1:320±)	+
7	*C. fatigans*	larva	(1:320±)	++
8	" "	pupa	(1:320±)	++++
	Control against Saline Solution			
9	Antiserum			0

B. *Against an Aquatic Insect Predator Extracted With Mosquito Pupae*

Before actually feeding immature mosquitoes to insect predators, it was desired to see if the presence of mosquito substance could be detected when the two organisms were simply extracted together.

Test 3. Three Hydrophilid larvae (Water Scavenger Beetle) were available for this test. One was extracted with one pupa of *A. quadrimaculatus,* another with three pupae of *C. restuans,* and the third after several hours of starvation. The greater number of culicine pupae was used since they appeared to be approximately one-third the size of the anopheline pupa. Two other test antigens were prepared from the two species of mosquitoes without the insect predator. The tubes were examined for rings after one hour at room temperature (table 3).

Results. A ring precipitate was formed in only those tubes that contained extracts of the mosquito pupae. The presence or absence of the Hydrophilid larval substance in the same extract did not seem to affect the intensity of the reaction (compare tube 1 with

Table 3—Precipitin Test with Anopheline Pupal Antiserum Against an Aquatic Insect Predator Extracted with Mosquito Pupae

Tube Number	Antigens: Organisms Extracted in 1.25 cc. Saline solution		Intensity of Rings After 1 hour
	Insect Predator	Mosquito	
1	Hydrophilid larva	1 *A. quadrimaculatus* pupa	++++
2	" "	3 *C. restuans* pupae	+++
3	" "	----------	0
4	----------	1 *A. quadrimaculatus* pupa	++++
5	----------	3 *C. restuans* pupae	+++
	Controls against saline solution		
6	Antiserum		0
7	Antigen (1 *A. quadrimaculatus* pupa)		0

tube 4, and tube 2 with tube 5). There was no cross-reaction between the antiserum and the Hydrophilid extract (a heterologous antigen). This would suggest that no proteins are in the larval stage of this predator that are antigenically similar to the anopheline pupal antigen; or that if similar proteins exist, they were not sufficiently concentrated in this particular test antigen to react with the antiserum. This same interpretation can be given to similar examples in later tests.

C. Against Aquatic Insect Predators Extracted After Starvation or After Feeding upon Larvae and Pupae of A. quadrimaculatus.

In the next two tests, various aquatic insect predators were collected from nature and brought into the laboratory. They were separated and starved over night. The next day, some of them were allowed to feed upon 4th stage larvae and pupae of *A. quadrimaculatus* for 30 to 60 minutes. They were then extracted in 1.25 cc. of saline solution after the removal of any conspicuous legs and wings.

Test 4. Two Water Scorpion adults (*Ranatra fusca*) and two Giant Walter Bug nymphs (*Belostoma sp.*) were used in this test. One specimen of each organism was allowed to feed for about 30 minutes. The Belostomas were quite large, probably the last nymphal stage. In addition to these, one test antigen was prepared from a pupa of *A. quadrimaculatus* (homologous antigen). Ring precipitin tests were set up and read after one hour at room temperature (table 4).

Table 4—Precipitin Test with Anopheline Pupal Antiserum Against Two Aquatic Insect Predators After Starvation or After Feeding upon Larvae and Pupae of *A. quadrimaculatus*.

Tube Number	Antigens: Organisms extracted in 1.25 cc. saline solution — Insect Predator	Immature *A. quad.* (4th stage larvae and pupae)	Intensity of rings after 1 hour
1	*Ranatra fusca,* adult	0 (starved)	0
2	*R. fusca,* adult; after eating	1 larva & 2 pupae	+++
3	*Belostoma* sp., nymph (large)	0 (starved)	0
4	*Belostoma* sp., nymph (large); after eating	1 larva & 1 pupa	+++
5	----------	1 pupa	++
	Controls against saline solution		
6	Antiserum		0
7, 8, 9	Antigens: same as 1, 2 and 4		0
10	Antigen: same as 3		±

Test 5. Nine species of aquatic insect predators were available for this test: two Giant Water Bug nymphs (*Belostoma sp.*), two Water Scorpion adults (*Ranatra fusca*), three Water Scavenger larvae (*Hydrophilids*), two Damsel Fly nymphs (*Zygoptera*), one Water Diving Beetle larva (*a Dytiscid*) another Water Diving Beetle larva (*Cybister sp.*), one Dragon Fly nymph (*Anax junius*), one May Fly nymph, and a Horse Fly larva. Although May Fly nymphs are mainly herbivorous, they have been cited by several authors as efficient predators of mosquitoes (see Hinman, 1934). Horse Fly larvae are carnivorous, but probably never have a chance to eat mosquitoes due to their sluggish activity. Specimens of the first five species mentioned above were allowed to feed on anopheline mosquitoes for 30 to 60 minutes and then extracts were prepared from their bodies. Extracts were also made from one pupa and one larva of *A. quadrimaculatus*. In setting up the protocol, antigen controls were included for all of the predators that had fed and for most of those that were starved. The readings after one hour are presented in table 5.

Results. In both tests 4 and 5, strong reactions occurred in all but one instance where the insect predator had fed upon anopheline larvae and pupae. The only example in which it failed to demonstrate the presence of ingested anopheline tissue was in the case of the Hydrophilid larva that had eaten only a small portion of a larval mosquito (test 5, tube 6). Most of the extracts of starved predators did not react with the antiserum (test 4, tube 1; test 5, tubes 1, 3, 5, 8, 11, 12, 14). According to these observations, those species revealing no antigenic similarity in the concentration tested were *Ranatra fusca* (adult), Hydrophilid (larva), Damsel Fly (nymph), Dragon Fly (nymph), and Tabanid (larva). A starved

Table 5—Precipitin Test with Anopheline Pupal Antiserum Against Several Aquatic Insect Predators After Starvation or After Feeding upon Larvae and Pupae of *A. quadrimaculatus*

Tube Number	Antignes: Organisms extracted in 1.25 cc. saline solution Insect Predator	Immature *A. quad* (4th stage larvae and pupae)	Intensity of rings after 1 hour
1	*Belostoma* sp., nymph (small)	0 (starved)	0
2	" " " " ; after eating	1 larva	+++
3	*Ranatra fusca*, adult	0 (starved)	0
4	" " " ; after eating	1 larva & 1 pupa	+++
5	Hydrophilid, larva	0 (starved)	0
6	" " ; after eating	small portion of 1 larva	0
7	" " ; after eating	1 larva	+++
8	Damsel fly nymph	0 (starved)	0
9	" " " ; after eating	1 larva	+
10	Dytiscid, larva; after eating	5 larvae & 1 pupa	+++
11	Tabanid, larva (large)	0 (starved)	0
12	May fly, nymph	0 (starved)	0
13	*Cybister*, larva (large)	0 (starved)	+++
14	*Anax junius*, nymph	0 (starved)	0
15	———	1 larva	+++
16	———	1 pupa	+++
	Controls against saline solution		
17	Antiserum		0
18-24	Antigens: same as 2, 7, 9, 11, 12, 14 and 16		0
25-27	Antegins: same as 4, 10 and 13		+

Belostoma gave a weak reaction in one instance (test 4, tube 3) and no reaction in another (test 5, tube 1). It should be pointed out that a large specimen was used in the first case and a smaller one in the second. This might indicate the ability of a cross reaction occurring when a sufficiently high concentration of a heterologous antigen is present. A much stronger cross reaction was obtained with the starved *Cybister* larva (test 5, tube 13). Here again the predator was large and constituted a great concentration of the antigenic substance. The only Dytiscid larva (other than the *Cybister*) available fed upon several mosquitoes. The extract prepared from this engorged predator gave a strong reaction (test 5, tube 10). However, since it is closely related to the *Cybister*, it would be expected that a starved specimen would also have given a cross reaction. Most of the antigen controls formed no precipitates with the saline solutions. The one antigen control in test 4 (tube 10) and the three in test 5 (tubes 25, 26, 27) that did not present noticible rings indicate that these test antigens contained some particulate matter that settled at the interphase of the two liquids. These slight precipitations in the controls should be considered as contributing somewhat to the strength of the reactions between these particular antigens and the antiserum.

D. Against Aquatic Insect Predators and Anopheline Larvae Collected in the Field.

Test 6. Sufficient time was available at the completion of the above tests to make one field application of this method of testing aquatic insect predators of mosquitoes. The necessary field equipment for collecting and extracting the organism is quite simple. It consisted of a dipper, pipettes, vials, small test tubes, glass rods, and a supply of sterile water. One and a quarter cc. of sterile normal saline solution was added to each of the test tubes before leaving the laboratory. All of the organisms were collected within a small area of a quiet pool. They were washed in the vials with several changes of sterile water. Then they were transferred to the test tubes and macerated in the 1.25 cc. of saline by means of a glass rod. The macerated organisms were at atmospheric temperature for 2 to 3 hours before being brought into the laboratory, and stored in the refrigerator for 48 hours. This delay before performing the test was unavoidable. Without any futher extracting, the contents of the test tubes were filtered and used for setting up the protocol. One fourth-stage larva of *A. quadrimaculatus* from the insectary was extracted in a similar manner and included in the test. After one hour at room temperature the series of small tubes was examined for the presence of rings (table 6).

Table 6—Precipitin Test with Anopheline Pupal Antiserum Against Aquatic Insect Predators and Anopheline Larvae Collected and Extracted in the Field

Tube Number	Antigens: Organisms extracted in 1.25 cc. saline solution		Intensity of Rings after 1 hour
	Insect Predators	Immature Anophelines	
1	*Belostoma*, nymph (large)		++
2	Damsel fly, nymph (large)		+
3	Damsel fly, nymph		0
4	Dragon fly, nymph		0
5	Hydrophilid, larva		0
6		4th stage (Green)	+++
7		4th stage (Green)	+++
8		*A. quad.*, pupa (from insectary)	++++
	Control against saline solution		
9	Antiserum		0

Results. Two of the five insect predators gave positive precipitin reactions with the anopheline pupal antiserum. The significance of these reactions with the nymphal stage of *Belostoma* and the Damsel Fly larva (tubes 1 and 2) cannot be adequately interpreted at the present development of the test. However, in the light of previous tests, it should be noted that these were relatively large specimens which may have accounted for the positive reactions.

Strong reactions occurred with all of the anopheline larval extracts (tubes 6, 7 and 8). The green anopheline larva (tube 6) had obviously eaten some chlorophyl bearing plant. It is interesting to observe that this particular food selection of the larva did not interfere with the reaction. The most important point demonstrated by this test is that anopheline larvae *extracted in the field* can react with anopheline antiserum.

Discussion

This preliminary investigation shows that it is possible to demonstrate by precipitin tests the presence of mosquito larvae and pupae in the digestive tracts of aquatic insect predators. However, since there were a few non-specific reactions there are several refinements that could be instituted that might improve the specificity of the test. In all of the experiments the antiserum was always diluted with one part of saline solution. No doubt if the antiserum had been used undiluted a higher titer could have been obtained perhaps without encountering the prozone phenomenon. A higher titer might make it possible to screen out the cross reactions between the anopheline antiserum and the culicine antigens. A better way of differentiating between engulfed anopheline and culicine proteins would probably be to develop a culicine antiserum and compare the reaction of the same test antigens with the two antisera.

Positive reactions with certain aquatic insects that have been starved might be difficult to avoid. Such cross reactions in the above tests were with relatively large predators. If antigenically similar proteins exist in these organisms, they perhaps represent only a small proportion of the entire insect body; however, when sufficiently concentrated, these proteins appear to be capable of reacting with the anopheline antiserum. Such reactions could perhaps be avoided by using higher dilutions of the test antigen with an anopheline antiserum of greater potency or, in some cases, it might be necessary to prepare the test antigen from the intestinal tract dissected from the predator.

The above tests were performed shortly after the predators had fed upon the mosquitoes. It is possible that as digestion progresses the reactions might become negative. King and Bull (1923) found that unsatisfactory results were obtained when precipitin tests were performed twelve to eighteen hours after the adults had taken blood meals.

The experiment in which the test antigens were prepared in the field demonstrates that under those particular conditions the extracts of the anopheline mosquitoes were capable of reacting with the anopheline antiserum. In that experiment the macerated organisms remained at atmospheric temperature (around 90° F.) for two

or three hours before being taken to the laboratory. It might be found necessary to refrigerate the extracts or preserve them with merthiolate while still in the field.

Although the present investigation concerned the destruction of immature mosquitoes by aquatic insect predators, the same technique no doubt could be applied to the study of insect predators of adult mosquitoes. Hinman (1934) cites a number of references of adult mosquitoes being destroyed by other insects.

This investigation was undertaken primarily from an academic interest for the authors are not inclined to attach great importance to the activity of aquatic insect predators as a practical method of controlling malaria. However, it does seem possible that the test might be used advantageously in studying the ecology of malaria vectors. Furthermore, it might be applied with greater significance to investigating the vectors of other diseases that are subject to more efficient predatory activities of insects.

Summary

1. An anopheline pupal antiserum was produced by intraperitoneal inoculations of a rabbit with macerated pupae of *A. quadrimaculatus*. This antiserum had a precipitin titer of over 1:4320 when tested against the homologous antigen.

2. The antiserum was found to react with the larval, pupal, and adult stages of both anopheline and culicine mosquitoes. There was some indication that it was slightly more specific for pupa than for species.

3. Six species of aquatic insect predators that had been starved over night gave no cross-reaction with the anopheline pupal antiserum (*Ranatra fusca* adult, a Hydrophilid larva, a Damsel Fly nymph, a May Fly nymph, *Anax junius* nymph and a Tabanid larva. Two others (*Belostoma sp* nymph and *Cybister sp.* larva) gave a slight reaction when tested in the same manner. The latter two specimens were relatives large organisms and produced concentrated test antigens.

4. Except in one instance, the precipitin test was capable of detecting that the insect predators (*Belostoma sp.* nymph, *Ranatra fusca* adult, Hydrophilid larva, Damsel Fly nymph, and perhaps Dytiscid larva) had recently fed upon immature mosquitoes. The one failure occurred when a Hydrophilid had eaten only a small portion of one larva.

5. One trial under field conditions demonstrated that immature anopheline mosquitoes could be extracted at the time of collection, and later would react with anopheline antiserum when brought into the laboratory.

6. This preliminary investigation indicates that a precipitin test might be used with advantage in the study of natural insect enemies of the vectors of malaria and other diseases.

References

Bull, Carrol G. and King, W. V.
1923 The Identification of the Blood Meal of Mosquitoes by Means of the Precipitin Test. Amer. Jour. Hyg., 3:491-496.

Flosdorf, Earl W. and Mudd, Stuart
1938 An Improved Procedure and Apparatus for Preservation of Sera, Microorganisms and other substances—The Cryochem Process. Jour. Immunol., 34:469-490.

Hess, A. D. and Tarzwell, Clarence W.
1942 The Feeding Habits of *Gambusia affinis affinis,* with Special Reference to the Malaria Mosquito, *Anopheles quadrimaculatus.* Amer. Jour. Hyg., 35:142-151.

Hildebrand, Samuel F.
1925 A study of the Top Minnow, *Gambusia holbrooki,* in its Relation to Mosquito Control. U. S. Pub. Health Bull. No. 153, Government Printing Office, Washington, D. C.

Hinman, E. Harold
1934 Predators of the Culicidae (Mosquitoes). I. The Predators of Larvae and Pupae, Exclusive of Fish. II. Predators of Adult Mosquitoes. Jour. Trop. Med. and Hgy., 37:129-134, 145-150.

King, W. V. and Bull, Carroll G.
1923 The Blood Feeding Habits of Malaria - Carrying Mosquitoes. Amer. Jour. Hyg., 3:497-513.

Russell, Paul F. and Jacob, V. P.
1939 Some Experiments in the Use of Fish to Control Anopheles Breeding in Casuarina - Pits. Jour. Malaria Insti. of India, 2:273-291.

According to our constitution, "Any person who has shown scientific or practical interest in malaria problems may be proposed for membership in the Society and may be elected to membership as provided in the by-laws of the Society."

Due to the rapidly growing interest in malaria the membership committee can hardly keep well informed of all persons working in this field and the specialties related to it. There may be many individuals whose membership in the Society would profit both the Society and themselves. It is suggested, therefore, that the members of the Society bring such persons to the notice of the membership committee, or submit application for membership for them.

Signed, the Membership Committee
John H. O'Neill
Arthur J. Walker
Wendell Gingrich, Chairman, Univ. of Texas Medical School, Galveston, Tex.

OBSERVATIONS ON THE CHARACTER OF THE PAROXYSM IN VIVAX MALARIA*

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(Received for publication 4 May 1945)

The use of malarial infections for over two decades as adjunctive therapy in neurosyphilis has provided invaluable opportunities for study of the plasmodial diseases. The physician in the field may see several thousand segments of malarial infections but rarely if ever encounters a patient in whom he may observe the uninterrupted evolution of a malarial attack. Few aspects of these diseases have been entirely ignored, yet many merit more thorough consideration than they have been accorded. In this paper the results obtained from an analysis of the febrile reactions of white patients to naturally acquired infection with *Plasmodium vivax* will be presented.

Material and Methods

The data to be described here were derived from therapeutic vivax infections in 70 patients with neurosyphilis. Attacks included were those which (1) were naturally induced with a single strain (McCoy) of *P. vivax*, (2) had terminated spontaneously and at no time has required plasmodicidal medication, and (3) were free from intercurrent infection (excepting syphilis) and other complications during the malarial attack. All attacks were characterized by continuous clinical activity with not more than two successive paroxysm-free days on any occasion between the first and last paroxysm.

The first 51 attacks have been divided into four groups on the basis of their duration: Group I with 15 attacks of one to 10 days; Group II, 15 attacks of 11 to 20 days; Group III, 10 attacks of 21 to 30 days, and Group IV, 11 attacks of over 30 (31 to 63) days. These 51 infections comprised 1,000 paroxysms or units of clinical activity. In Group V are included 11 attacks which were induced by inoculation of patients from eight to 11 hours later in the day than those of Groups I to IV. Group VI comprised attacks of eight additional patients that served only as a source of data for 50 paroxysms whose rigors commenced with temperatures of 100° F. or more.

Four-hourly oral temperature records (day and night) are kept for all patients on the malaria therapy service, starting on or before the day of inoculation. For practical reasons 100° F. has been arbi-

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation, in cooperation with the Florida State Board of Health and the Florida State Hospital.

trarily chosen as the baseline of the febrile range for the paroxysm. Temperature elevations of lesser degree do occur prior to clinical activity of unquestionably malarial origin, but similar elevations of non-malarial origins are common, particularly in psychotic patients. If the temperature is found to have reached 100° F., or a rigor is commencing, or the patient complains of other symptoms which suggest the onset of a paroxysm, the temperature is taken hourly as long as it is elevated. The highest point observed is recorded as the "maximum observed temperature." The hour of the day and temperature at both the onset and the termination of rigor are also recorded.

Observations have been made on the clinically active phase of the infections, as well as its natural subdivisions. We have examined the types of onset and character of the attacks; the incidence of rigoriferous and rigorless paroxysms; the pyrogenic and the rigor-inducing parasite densities; characteristic of the rigors, duration of component parts of the paroxysm; the time of their occurrence; the relation of this timing for successive paroxysms of the same cycle as the disease progressed. For the latter purpose two cycles whose paroxysms are responsible for the quotidian fever are designated: *A*, the one whose paroxysms fall on the odd days, and *B*, the one whose paroxysms fall on the even days from the day of inoculation.

Statistical treatment of the data has been simple. Standard errors have been calculated for means, and differences exceeding twice the value of the standard error have generally been accepted as significant. Tests of variance have been made when comparison of means descriptive of a given attribute in several groups was required. The Chi-square test has been applied to determine whether two or more distributions have come from the same population. The coefficient of correlation has been used to determine the degree of association between two attributes.

Observations

Detailed consideration of the incubation (days to first fever) and prepatent (days to first parasites) periods have been omitted from this study. The former varied from nine to 23 days (mean, 13.14 ± 0.42 days, and the latter from nine to 22 days (mean 11.67 ± 0.33 days). In five of the 51 attacks the initial fever preceded the first detection of parasites; both events took place on the same day in 10 instances; and in the remaining 36 attacks the prepatent period was from one to seven days shorter than the incubation period. The first appearance of parasites preceded the clinical onset in all 15 of the shorter attacks of Group I. The mean

period of precedence of first parasites over initial fever for the 51 attacks was 1·47 ± 0.24 days.

Onset, Type and Segments of the Attack

Although the last few days of the incubation period are commonly characterized by such premonitory complaints as headache, lassitude, vague aches and pains, chilly sensations, anorexia, and general malaise, such symptoms are not necessarily pathognomonic. The initial febrile reaction to multiplication of the plasmodium ushers in the clinical attack proper.

Figure 1 shows that clinical activity may be introduced by either intermittent or remittent fever, or by a combination of both. The first few days of the attack may be distinguished by (1) paroxysms at regular quotidian or tertian intervals; (2) a continuous fever lasting as long as three or four days, which may be characterized by 24-hourly peaks between which the temperature may fall below 100° but not to 98.6°; or (3) one or two discrete intermittent paroxysms followed by 24 to 72 hours of remittency as described under (2) above.

Table 1 shows the variety of types of onset in 50 of the attacks. Of these, 36 began with either quotidian or tertian intermittent activity, and interestingly enough all but one of the Group I attacks were included here. Of the 14 attacks that exhibited remittent fever at some time during the first week, all eventually assumed a quotidian intermittent character. Of the eight attacks that began with

Table 1—Mode of Onset and Type of Subsequent Attack in 50* Vivax Infections

Mode of onset	Clinical type of attack														Tertian Intermittent	Total No.	Total Per cent
	From onset	Quotidian intermittent									Tertian Intermittent	Total No.	Total Per cent				
		After tertian intermittency of			After intermittency and remittency of			After remittency of									
		3 days	5 days	7 days	4 days	5 days	6 days	2 days	3 days								
Intermittent Quotidian	28															28	56
Tertian		4	1	2							1					8	16
Intermittent remittent					3	3	1									7	14
Remittent								4	3							7	14
Total	28	4	1	2	3	3	1	4	3		1					50	
Per cent	56	14			14			14			2					100	

* One attack (Group I) comprising a single paroxysm is omitted from consideration.

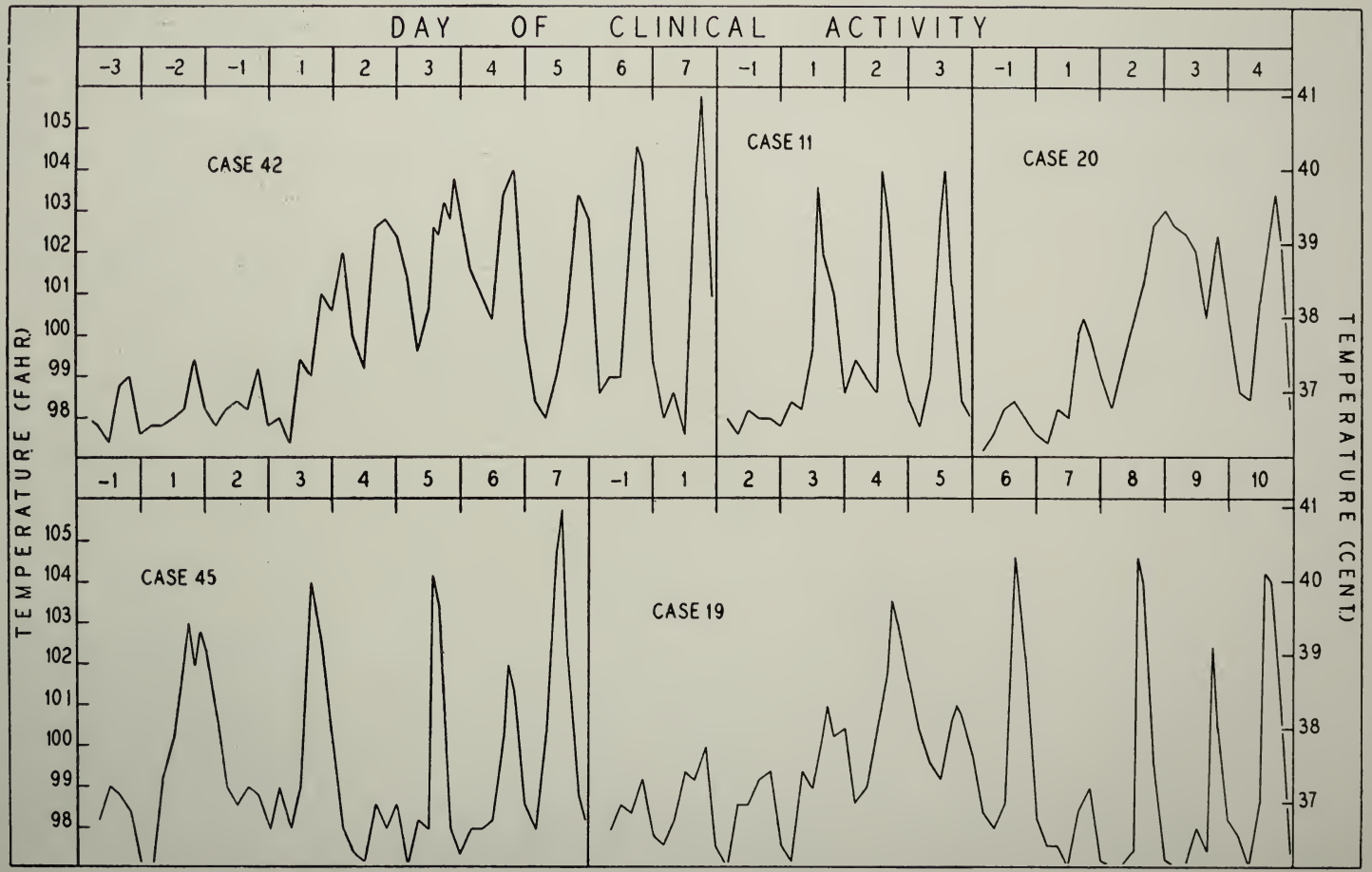

Fig. 1—Different types of febrile reaction accompanying initial clinical activity.

tertian intermittency, a second brood of parasites developed in seven and converted the clinical picture to that of quotidian intermittency. Only one of the 50 infections pursued a tertian course throughout the attack.

Remittent fever, when present, is identified with the initial phase of the attack and is apparently caused by unsynchronized sporulation of the plasmodium. When the parasites become regimented into two broods, the remittency, as Figure 1 shows, gives way abruptly to quotidian intermittent fever. At this stage the paroxysms are distinguished by sharp febrile peaks, and a rapid return of the temperature to the normal zone which creates a fever curve with a comparatively narrow base. This baseline, however, is not as low or as narrow during the first few intermittent paroxysms as it is later.

Certain key points have been designated as natural boundaries of portions of the malarial infection. Mean durations were computed for each segment and are shown in Figure 2 and Table 2. Data apply to 49 patients only, since two did not experience any rigors during their attacks. The characteristics of the means of the separate groups may be summarized as follows:

Group I: These were the patients with attacks of 10 days or less. First parasites appeared late and the interval from first parasites to first fever was definitely longer. Rigor occurred later in relation to point of inoculation, and subsequent intervals were shorter for patients in this group. The mean duration of attack was about half that of the next group, as it should have been in view of the manner of selecting the groups.

Group II. The mean interval from inoculation to first fever was three days shorter than that for patients in Group I. All intervals following onset were longer, however.

Group III. The sum of the segments of attacks over three weeks in length resembled that in Group I up to the onset of rigor. From that time on differences were great and are, of course, related to the greater duration of the attack. While rigor appeared at about the same time, more than six days elapsed before parasites reached their maximum density and nearly 14 days from that point to the day of final rigor.

Group IV. These patients with attacks up to 63 days in duration had a mean incubation period similar to that for patients in Group II whose attacks were only one-third that length. Group IV means did not differ materially from those of earlier ones until rigor set in. Following this point they were longer. The mean interval from maximum parasites to last rigor (26.4 ± 3.3 days) was twice

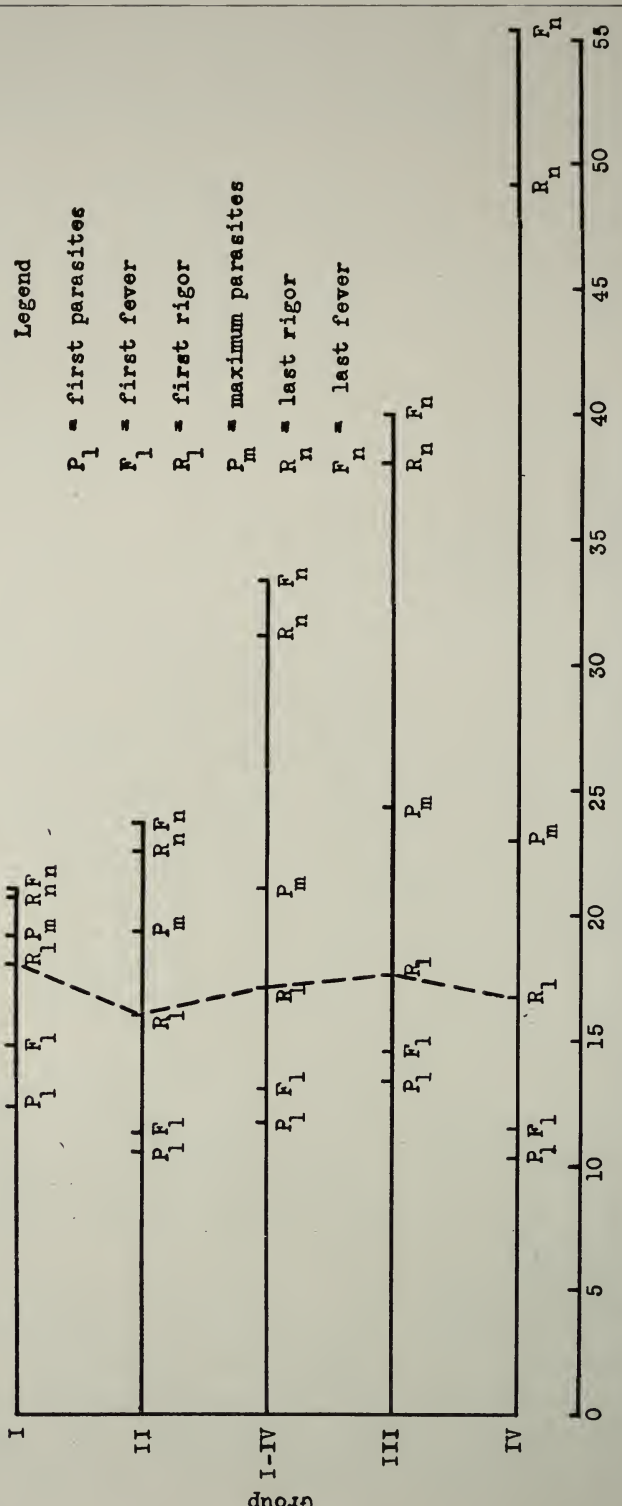

Fig. 2—The mean day of occurrence of key events of the attacks.

Table 2—Mean Duration in Days of Segments Composing the Primary Vivax Attack with Standard Errors

Intervals	Group I	Group II	Group III	Group IV	Total	Standard deviations
Number of Patients	13	15	10	11	49	
First fever to first rigor ($F_1 - R_1$)	4.15 ± 0.62	5.67 ± 0.57	4.10 ± 0.70	6.27 ± 0.67	5.08 ± 0.32	2.206
First rigor to maximum parasites ($R_1 - P_m$)	1.23 ± 1.02	3.33 ± 0.95	6.60 ± 1.17	6.18 ± 1.11	4.08 ± 0.53	3.685
Maximum parasites to last rigor ($P_m - R_n$)	1.54 ± 2.99	3.33 ±2.79	13.70 ± 3.41	26.36 ± 3.26	10.14 ± 1.54	10.797
Last rigor to last fever ($R_n - F_n$)	0.38 ± 0.93	1.13 ± 0.87	1.90 ± 1.06	6.00 ± 1.01	2.18 ± 0.48	3.352
Total duration of attack	7.31 ± 4.26	13.46 ± 3.97	26.30 ± 4.86	44.82 ± 4.64	21.49 ± 2.20	15.377

* Standard errors were calculated from the standard deviations of the respective interval.

that for the previous group.

The point of departure for these four groups of patients appears to have occurred with the onset of rigor (Figure 2). There was a maximum of two days' difference in the sum of the mean intervals up to this point. The greatest group differences are found in the time elapsing between the day of maximum parasite density and the day of last rigor.

The Paroxysm

Our practice has been to define the malarial paroxysm as that periodic elevation of oral temperature to 100° F. or more which is attendant upon sporulation of the plasmodium, and which may or may not be preceded, or accompanied, by a rigor ("chill").

The classical description of the paroxysm recognizes three stages, namely, the cold (rigor or chilly sensations), the hot (fever), and defervescent (diaphoresis). The patient usually receives some warning of the impending rigor from two or three minutes up to half an hour beforehand, consisting of vague chilly sensations, possibly headache, nausea, and general localized aches and pains. Commonly his temperature commences to rise during the cold stage and continues to do so until the peak is reached a few hours later. Before the fever subsides, diaphoresis becomes evident and may continue for two or three hours. The patient finally, usually from five to ten hours after the onset of the rigor, regains a more or less comfortable, though variably weakened state.

Experience has taught us, however, that the paroxysm frequently is not as clear in regard to its component parts as the textbook descriptions indicate. As a matter of fact, the only indispensable attribute is the elevated temperature. The rigor may be wanting; and the cold phase, if represented at all, may be marked by chilly sensations. Certain characteristics of the true chill as, for example, the pale and pinched facies, the shivering, the lividity that is usually evident in the lips and finger nails, and cutis anserina ("goose-flesh" or "chill bumps") may not be observed. Other uncommon varieties of the classical accounts occur. Occasionally the rigor does not commence until the fever is at its peak, or the temperature at the end of the rigor may have fallen close to, or within, normal range. On the other hand the rigor may start and terminate while the temperature remains below 100°, the fever delaying its appearance for an hour or more. Infrequently, too, the rigor may be divided into two parts by an intermission during which the patient ceases shaking. Very rarely, the peak temperature may be reached during rather than following the rigor. The fever, unheralded by premonitory sensations in some instances, may be so slight as to

escape the attention of the patient. The diaphoresis in the third stage of the paroxysms may vary from the one extreme in which the bed clothes are saturated with perspiration to the other of unnoticeable degree.

While the genesis of the paroxysm is, of course, intimately associated with the rupture of the mature schizont and release of the merozoites, the exact pyrogenic factor is not known. The most general assumption is that it represents the body's reaction to foreign protein released at the time of sporulation.

In our series of attacks the initial paroxysm appeared at any time from two days before to seven days after parasites were first detected. Obviously, then, the number of parasites per cubic millimeter of peripheral blood at the onset of clinical activity, which may be termed the pyrogenic density of parasites, must vary considerably from person to person. Examination showed that the patients of Group I, with short attacks, had a higher mean pyrogenic threshold than did those with the longer attacks, but the differences between mean densities for the groups in this study were not significant.

Although the number of paroxysms was found to be correlated with the duration of clinical activity, a certain number of fever-free days were noted between the first and last paroxysms of the attacks. The normal clinical picture is here regarded as essentially quotidian. The missing paroxysms (fever-free days) have been divided into two groups. Both are illustrated in the febrile curve of Patient No. 19, Figure 1. This person's attack opened on the odd day, Cycle *A* paroxysm. Cycle *B* paroxysm did not appear until the fourth day, leaving the patient afebrile on the second day. Also the Cycle *A* paroxysm expected on the seventh day failed to appear. The latter, *intracyclic,* failure belongs to those which occur between the first and last paroxysms of a cycle after it has been established. They are true instances of missed paroxysms, and the reason for them is not readily apparent where there is no associated drop in the parasite density.

The failures in the extracyclic group occur when only one cycle is in operation (tertian activity), usually at the beginning or end of the attack. These failures are attributed to the cycle whose paroxysms would have occurred on those days and are due (1) to the failure of one brood of parasites to attain pyrogenic density as soon as the other, or (2) in terminal failure, to subsidence of the density of one brood of parasites below pyrogenic level before that of the other. The majority of missing paroxysms in our data were intracyclic in type (55.2 per cent). In the extracyclic group more (69.2 per cent) of the failures were odd-day, or Cycle *A*, paroxysms, where-

as those of the intracyclic group were equally divided between Cycle *A* and *B*.

The rigor

Variation in the incidence of rigor occurred in relation to both the stage and duration of the attack. The mean parasite density required to initiate rigor, 4,012 ± 669 per cmm., was significantly greater than the mean pyrogenic density, 274 ± 140 per cmm. Hence the first few paroxysms of an attack in the more susceptible person were usually rigorless, as illustrated in Table 2 by the interval between first fever and first rigor. The differences between groups in the length of this interval were insignificant, but the mean duration for all attacks was 5.08 ± 0.32 days. Table 3 presents the incidence of rigor according to group. Although two-thirds of all paroxysms exhibited rigor, the shorter attacks of Groups I and II showed an incidence of 51.2 per cent of rigoriferous paroxysms as opposed to 71.1 per cent for the longer attacks of Groups III and IV.

Table 3—Frequency of Rigor among Paroxysms

Group	Paroxysms		
	Total	With rigor Number	Per cent with standard error
I	97	45	46.4 ± 5.06
II	190	102	53.7 ± 3.61
III	246	188	76.4 ± 2.71
IV	467	319	68.3 ± 2.15
Total	1000	654	65.4 ± 1.50

As the above-mentioned characteristics of the rigor varied, so also did the temperature at onset and the duration. In a random sample of 250 rigors from Groups I to IV the temperature at onset was less than 100° in 71.6 per cent. It was less than 98.6° in 29 per cent, and the mean for all onsets was 99.5°.

The mean duration of all 654 rigors in Groups I to IV was 52.3 ± 0.7 minutes and, as a rule, at some point during the rigor the temperature began to rise. A random sample of 275 of the 654 rigors showed temperature increases of 0 to 7.2° from onset to termination. Temperature excursions from 3.0° to 4.8° were noted in over half of the rigors. More than 35 per cent of those in Groups I and II exhibited a range of four degrees, while of the patients in Groups III and IV almost as many had a five- as a four-degree range. Mean group values are shown in Table 4. The variance test applied to these mean ranges indicated that those for Groups I and II were significantly lower than the mean range for the total ($P<0.01$).

Febrile phase of the paroxysm.

The maximum temperature. Once the temperature starts to

rise during the cold phase, it continues upward to a maximum point which is reached in some instances at the termination of rigor but in most paroxysms sometime later. The maximum temperatures observed during the 1,000 paroxysms in Groups I to IV are presented in Table 5, classified on the basis of presence or absence of rigor. The variation among the group means was significant, and the presence of rigor was associated with a mean maximum temperature that was significantly higher ($P<0.01$).

Table 4—Mean Temperature Range during 275 Rigors

Group	Number of rigors	Mean temperature range with standard errors in degrees F.*
I	36	3.7 ± 0.22
II	36	3.5 ± 0.22
III	104	4.4 ± 0.13
IV	99	4.4 ± 0.13
Total	275	4.2 ± 0.08

*Standard deviation (1.2968°) given by residual variance was used to compute the standard errors.

The highest temperatures noted in the course of the attack were not usually attained during the first few paroxysms. This is clearly brought out in Figure 3, where the mean maximum temperatures are charted by group and by day during the first 10 days of the attack. The peak average temperature for all four groups combined occurred on the seventh day, as did those for Groups I, II, and

Table 5—Mean Maximum Observed Temperatures for 1000 Paroxysms

	Mean maximum temperature with standard error in degrees F.*		
Group	Rigor+	Rigor−	Total
I	104.0 ± 0.15	102.4 ± 0.14	103.1 ± 0.10
II	104.4 ± 0.10	102.3 ± 0.11	103.4 ± 0.07
III	104.4 ± 0.07	102.7 ± 0.13	104.0 ± 0.06
IV	104.2 ± 0.06	102.6 ± 0.08	103.6 ± 0.05
Total	104.2 ± 0.04	102.5 ± 0.05	103.6 ± 0.03

Standard deviation (1.0013°) based on residual variance was used to compute standard errors.

IV individually. The highest mean of Group III attacks was attained on the tenth day and maintained on the eleventh. In none of these groups did the mean maximum temperature reach subsequent levels as high as those on the seventh to tenth days.

The hour of occurrence. — The hour of occurrence of the paroxysms must designate a point common to all. In rigoriferous paroxysms the time of onset of the rigor would be a satisfactory one. In the absence of rigor, however, the exact time at which the paroxysm begins cannot be determined, and the first elevation of temperature to 100° is not comparable to the onset of rigor. The hour of occurrence of the observed maximum temperature has, therefore,

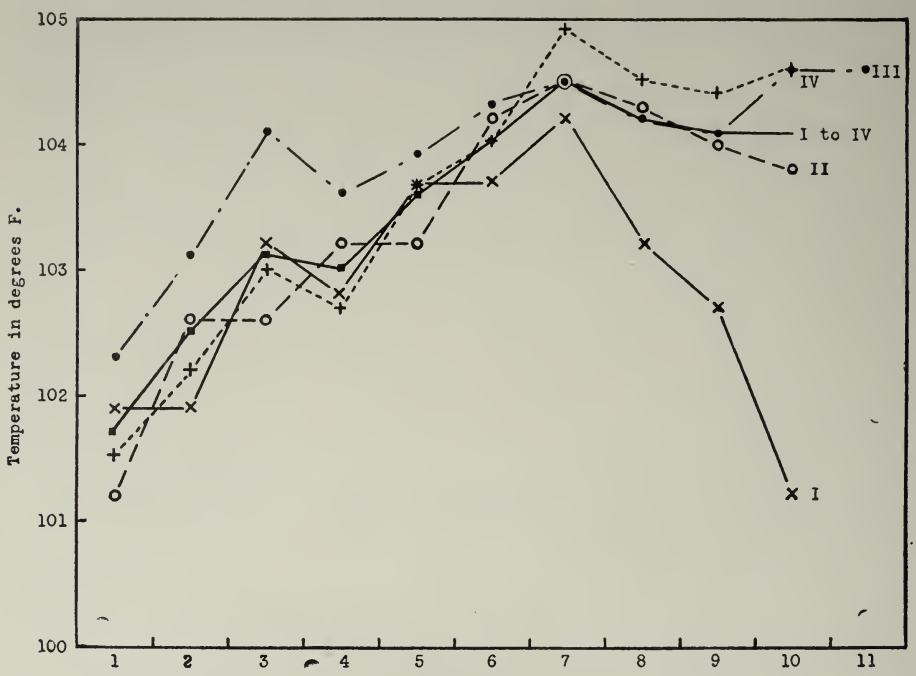

Fig. 3—Mean maximum observed temperature of paroxysms by day and by group during the first ten days of the attack.

been selected for the purposes of comparison.

The distribution of the 1,000 paroxysms of the 51 attacks according to hour of maximum temperature is compared in Figure 4 with that of the 250 paroxysms of Group V. The largest proportion of paroxysms in Groups I to IV occurred at 4 P. M. (17.3 per cent) and nearly two-thirds (64.9 per cent) were found in the six hourly observations from 3 to 8 P. M., inclusive. Only 8.7 per cent occurred during the hours 1 A. M. to 12 M., inclusive, while no maximum temperature were recorded at 7 or 8 A. M. in these groups.

The patients in Group V were those inoculated in the evening, and Figure 4 shows major peaks for these paroxysms at 4 and 8 P. M. with a minor rise at 2 and 4 A. M. When, however, the distribution of Group III paroxysms which followed inoculation at a morning hour was compared with that for Group V, the difference was negligible. It cannot be stated unequivocally that the later hour of inoculation influenced the hour of maximum temperatures of the paroxysms in Group V.

The distributions by hour of maximum temperature for the 654 rigoriferous and the 346 rigorless paroxysms are shown in Figure 5. The major peak of both curves occurred at 4 P. M., and while this was the only peak for the paroxysms with rigor the rigor-

less paroxysms rose to a second peak at 8 P. M. and possibly to a third at 12 P. M.

These subsidiary peaks may be explainable as follows: Temperature readings are made every hour during a rigoriferous paroxysm so that the rise and fall of the fever is fairly well described. The onset of the rigorless paroxysms is not well defined and many are probably first detected on the routine (four-hourly) temperature observations. The maximum observed temperature may truly occur at 8 or 12 P. M. in some paroxysms, while in others the peak may have been reached previously, but the 8 or 12 o'clock reading will be the maximum recorded. Thus there is an accentuated frequency of maximum temperatures recorded when the four-hourly *post meri-*

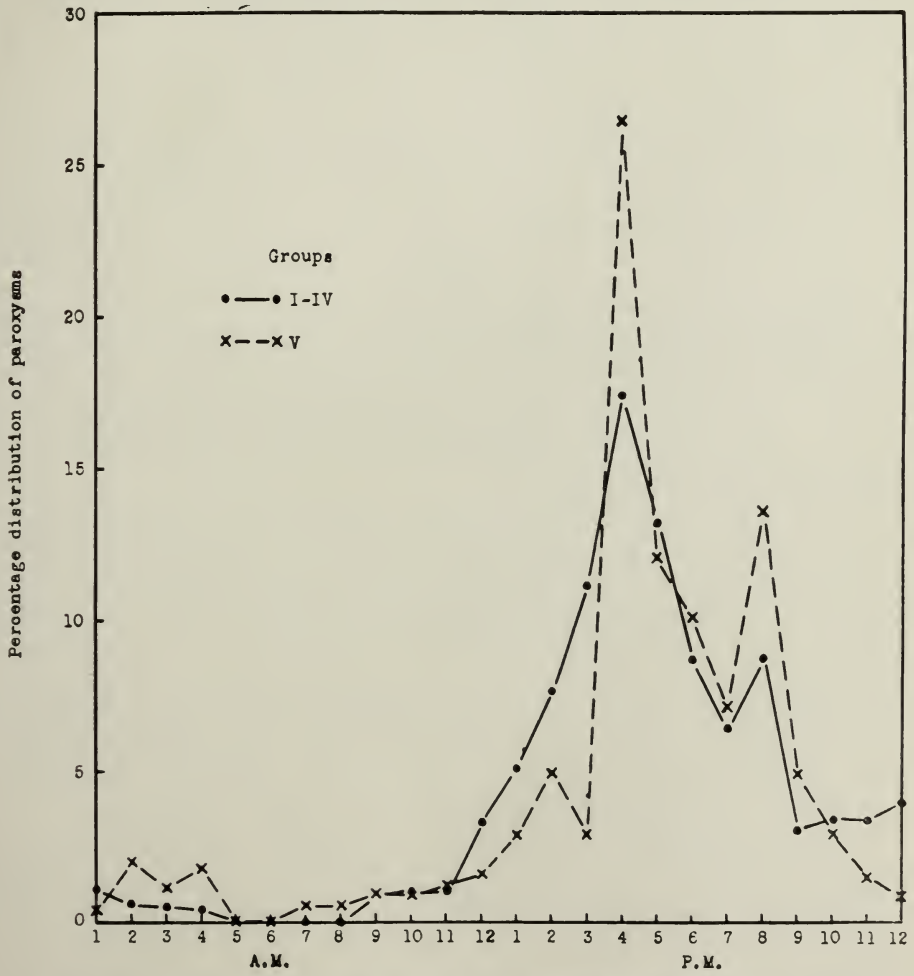

Fig. 4—Distribution according to hour of maximum temperature of 1000 paroxysms in groups I-IV and of 250 paroxysms of group V.

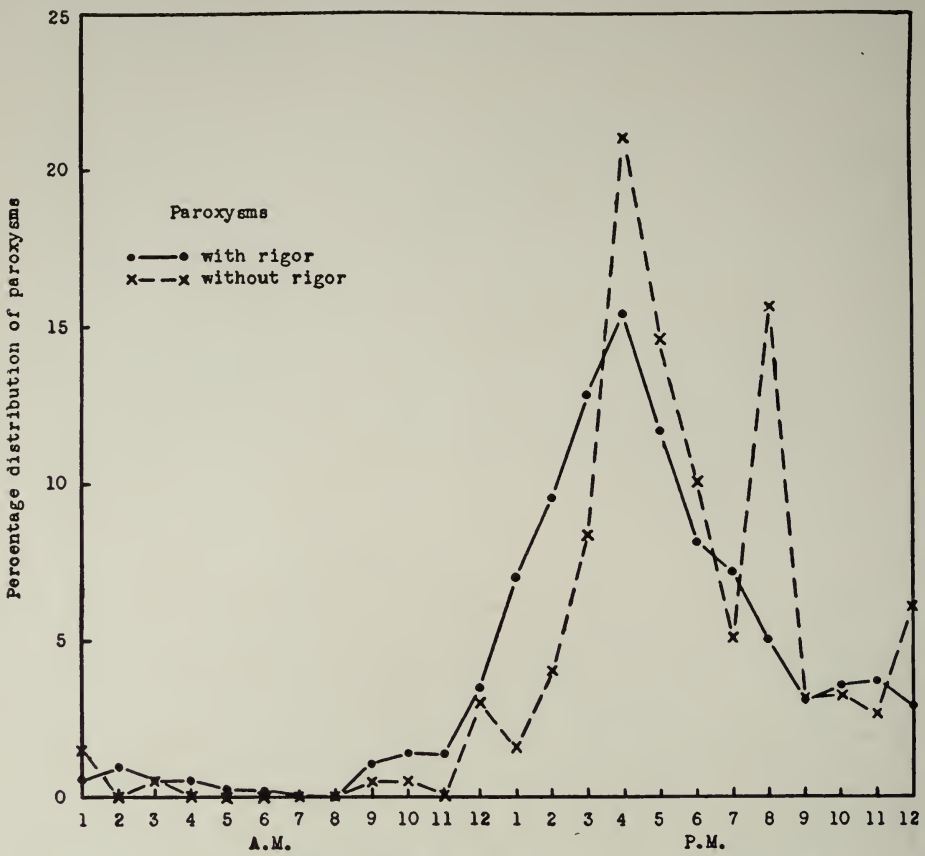

Fig. 5—Distribution of rigoriferous and rigorless paroxysms according to hour of maximum observed temperature. Group I-IV combined.

dian observations are made.

The mean hours of maximum temperature of the paroxysms for each group and according to the presence or absence of rigor are shown in Table 6. The group means differed significantly, and the mean for all observations was 4:39 P. M., which was very close to that for Group V paroxysms (4:25 P. M.) This corroborates the earlier evidence that the later hour of inoculation did not materially alter the time of occurrence of the paroxysms. Rigorless paroxysms occurred later in the day than did those with rigor. In Group III the difference between the two means was more than three and one-half hours. Group IV means alone did not differ significantly.

The distribution of the 654 rigors according to hour of onset is shown in Table 7. Here the mean hours of onset probably differed significantly ($0.05>P>0.01$) and may be compared with those of maximum temperature for rigoriferous paroxysms in Table 6. The differences for the various groups are from an hour and a half to two hours.

Anticipation versus postponement. The textbooks usually state that paroxysms occur at the same hour each day, although a few authors have called attention to some degree of anticipation (occurring at an earlier hour) or of postponement (falling somewhat later) of the paroxysm. The paroxysms of Group I and IV have been studied from the standpoint of their tendency to anticipate, postpone, or occur at the same time as their immediate predecessors of the same cycle. Table 8 contains the pertinent data showing the number of paroxysms which anticipated (or postponed) one, two,

Table 6—Mean Hour of Paroxysms at Maximum Observed Temperatures By Presence or Absence of Rigor

Group	Mean hour (P. M.) with standard error in minutes Rigor +	Rigor −	Total
I	2:40 ± 34	5:18 ± 31	4:05 ± 23
II	3:11 ± 22	5:53 ± 24	4:26 ± 16
III	2:31 ± 17	6:12 ± 30	3:23 ± 14
IV	5:44 ± 13	5:06 ± 19	5:32 ± 10
Total	4:12 ± 9	5:31 ± 12	4:39 ± 7
V			4:28 ± 14

*Standard deviation (3.7811 hours) based on residual variance was used to compute standard errors.

or three, etc. hours, as well as the proportion of hours of anticipation (or postponement) for each group.

Inspection of the data in Table 8 shows that a relatively large proportion of the paroxysms in Groups I and II were found to anticipate by from four to eight hours. A somewhat higher proportion

Table 7—Hour of Onset during Day of Rigors

Group	Mean hour (P. M.) with standard error in minutes*
I	12:47 ± 31
II	2:36 ± 20
III	1:40 ± 15
IV	4:14 ± 11
Total	3:00 ± 8

*Standard deviation (3.4152 hours) based on residual variance was used to compute standard error.

of Group IV paroxysms anticipated one hour than was observed in the other groups. Intergroup differences between distributions were significant ($P<0.01$).

The data in Table 8 also indicate that the proportion of paroxysms occurring at the same time increased from group to group, while from Groups I to III the percentage of paroxysms anticipating decreased and that of the paroxysms postponing increased. Group IV paroxysms upset these progressions by exhibiting a smaller proportion postponing. In general, more paroxysms anticipated than postponed, and the proportion of isochronic paroxysms was less than that of those postponing. The balance arrived at by these processes is

represented by the mean interval between febrile peaks of the same paroxysm cycle. This was found to be 47 hours and 44 minutes for the 900 intervals examined.

When the paroxysms were arranged with reference to hour of maximum temperature and day of disease it was found that hours of anticipation were more numerous during the first week of the

Table 8—Paroxysms, Showing Anticipation, Isochronism, and Postponent by Hours

	Anticipation					Isochronism		Postponement				Total
Group	Hours				%	Paroxysms	Per cent	Hours				Per cent
	4 - 8	3	2	1				1	2	3 - 8		
I	10	9	10	14	62	7	10	11	3	5	28	69
II	20	13	15	29	48	33	21	23	14	13	31	160
III	7	10	17	45	35	64	28	49	18	16	37	226
IV	15	14	28	103	36	157	35	83	34	11	29	445
Total	52	46	70	191	40	261	29	166	69	45	31	900*

*Fifty attacks are involved. One attack in Group I comprised but one paroxysm and was therefore omitted. One other patient in Group I exhibited a single cycle (tertian course) throughout, and this necessitated the elimination of only the initial paroxysm. The first paroxysm of each of the two cycles of the remaining 49 attacks could not be considered here since all paroxysms classified in regard to anticipation or postponement require a predecessor of the homologous cycle on which to base such classification. The total number of paroxysms included in the data in Table 8 therefore, is reduced to 900.

attack and those of postponement usually greater thereafter, but the tendency to anticipate was so pronounced in the early stages of the attack that there was significant overall negative correlation for each patient group between the hour of occurrence of the paroxysm and the day of disease.

Interestingly enough it was the rigorless paroxysms that anticipated significantly in the attacks of patients in Groups I and II. The correlation coefficients were -0.550, $P<0.01$, and -0.583, $P<0.01$, respectively. The amount of association between these two events was less for patients in Groups III and IV, but the correlation was still negative and still significant.

Rigoriferous paroxysms usually followed those without rigor and occurred earlier in the day (Table 6). They constituted the majority in attacks of Groups II to IV. They, too, anticipated somewhat but not to the same extent as did paroxysms without rigor. There was also definite evidence of postponement in the rigoriferous paroxysms of Group IV.

Segments of the Paroxysm

A study has been made of the length of certain phases of the paroxysm and of the temperature reactions associated with them. In this analysis the material comprised 123 paroxysms from Groups I to IV whose rigors commenced with temperatures below 100° together with 86 whose temperatures at onset of rigor were 100° or

over. The latter were taken from Groups I to IV (36) and from Group VI (50).

The period of the rigoriferous paroxysm has been divided into three segments as follows: segment A covers the duration of rigor, segment B the period from the termination of rigor to maximum observed temperature, and segment C the interval during which the temperature drops from peak to normal level.

Table 9—Segmental Means for Paroxysms Classified on basis of Temperature at Onset of Rigor

Temperature (°F.) at outset of rigor	Number of paroxysms	Segmental means in hours (H.) and/or minutes (M.) with standard errors			
		Segment A	Segment B	Segment C	Total A+B+C
97 - 99.8	123	53.4M ±1.5M	50.9M ±4.0M	6H, 54M ±11.0M	8 H, 38M ±11.0M
100 - 100.8	50	50.1M ±2.4M	48.9M ±6.2M	6H, 59M ±17.2M	8 H, 38M ±17.3M
101 - 101.8	25	40.2M ±3.4M	44.6M ±8.8M	7H, 29M ±24.4M	8 H, 54M ±24.5M
102+	11	58.6M ±5.1M	12.7M ±13.3M	8H, 42M ±36.7M	9 H, 54M ±35.9M
Total	209	51.3M ±1.2M	47.6M ±3.0M	7H, 31M ±8.4M	8 H 44M ±8.5M
Standard deviation *(M)		16.954	43.997	121.88	122.28

*Standard errors were calculated from the standard deviations of the respective interval.

It was found that the mean lengths of Group I and II paroxysms which began with a temperature under 100° were identical and were more than one hour longer than similar paroxysms for Groups III and IV. The mean segments for the 209 paroxysms, classified according to the temperature at onset of rigor, are shown in Table 9. The average length of the whole paroxysm remained fairly constant for those with onset at temperatures under 102°. The 11 paroxysms with onset at 102° or above, however, had a mean duration that was an hour longer.

Only segment C exhibited an increase in length paralleling that of the whole paroxysm. Segment B (the interval from termination of rigor to peak temperature) showed an inverse trend as did segment A (duration of rigor) for temperatures at onset under 102°

The temperature observed at the peak was positively correlated with that at the onset of rigor ($r=+0.308$, $P<0.01$). Also the correlation between temperature at onset and at the end of rigor was positive and significant ($r=+0.297$, $P<0.01$). This explains the brief duration of segment B in the 11 paroxysms initiated by a temperature of 102° or more, in eight of which the febrile reaction had reached its peak at the termination of rigor so that segment B became zero in length.

It has been observed that paroxysms near the beginning of the

attacks are generally longer than those occurring later. To illustrate this, the mean duration of the paroxysms of four attacks has been examined by segment, two from Group III and two from Group IV. There were 40 paroxysms in the first half and 43 in the second half. The mean duration of the paroxysms of the first half was nine hours, as compared with seven hours for those of the second half. The difference lay wholly in the greater length of segment c (from peak to normal temperature.)

Discussion

This study considerably amplifies some observations on vivax paroxysms made by one of us (S. F. K.), and subsequently summarized by Stratman-Thomas (1941). In the main the present findings corroborate the earlier ones. Recently Coatney and Young (1942) published data concerning the paroxysms in 21 patients infected with the St. Elizabeth strain of *P. vivax*. Their data disagreed with ours in certain respects. They described (1) a 55-minute period of fever prior to the onset of rigor, (2) a mean duration of 39 minutes for rigor, and (3) a mean temperature increase of 2 - 3° during rigor. Over 70 per cent of rigors in our series began while the patients' temperature was below 100°, and it was below 98.6° in 29 per cent. Our mean duration for rigors was 52 minutes, and our mean temperature range was 4.2°. The nature of these differences raises the question as to whether the authors observed the beginning of rigors. Too, they do not state the mode of infection. This is important, as the clinical reactions of patients following artificially induced infections cannot be accepted as representative of the natural attack.

In endemically malarious areas one encounters individuals who have experienced previous vivax infection, and such persons have relatively short attacks, often well under three weeks in length. Many patients inoculated for therapeutic purposes, however, are highly susceptible to the parasites and may experience two or more months of continuous clinical activity. The 51 infections in this series, grouped according to length of attack, therefore presumably represented different degrees of resistance.

The difference in days between the length of the incubation and the prepatent periods also is dependent upon the patient's resistance. In the most susceptible persons the initial paroxysm may appear before parasites can be demonstrated in the blood smears, while in those who have experienced a recent vivax infection, the parasitemia may precede the clinical onset by a week or more, during which time the density of parasites may increase to a pyrogenic level of several thousand per cubic millimeter. It is the patient whose clinical activity begins prior to the appearance of parasites who poses

a problem in diagnosis, inasmuch as the attack is likely to open with the continuous-remittent type of fever. Consequently, in an endemic zone one should not accept a single negative blood report as final, especially if the patient is a new arrival from a non-endemic area.

Many students of malariology who visit this station express surprise at the high incidence of quotidian febrile reaction among our vivax-infected patients. Either through reading or oral instruction they had been led to expect tertian periodicity. Explanations of quotidian activity given them were such as, "the patient was bitten by more than one infected mosquito," or "the patient was bitten on two successive nights by infected anophelines." We have observed, as did James (1926) that the bite of one infected anopheline on a single occasion can induce an attack characterized by quotidian paroxysms. Among the 51 attacks in this series, only one was tertian in character throughout. For some reason which we are unable to explain the parasites became divided into two broods whose maturation and sporulation times are separated by approximately 24 hours, thus creating quotidian activity. This presence of two broods may represent a compromise between the invasiveness of the parasites and the body's defense mechanism.

Discharge by the mosquito of sporozoites into the host's blood stream is assumed to be the important factor in the mechanism of infection. What part is played by the simultaneous deposit of sporozoites in the subcutaneous tissue in modifying the attack we do not know. The paroxysms due to the two parasite broods do not always appear within 24 hours of each other at the onset of the attack, but those caused by the more slowly developing brood usually appear within a week, thereby converting tertian activity to quotidian. Similarly, attacks frequently end with tertian periodicity because the numbers of one brood drop below pyrogenic levels more than 24 hours before the other. On the assumption that there might be some difference between the attributes of the paroxysms of the two broods, the data were classified by cycle and examined for all aspects studied in this investigation. The findings were wholly negative.

The belief that malarial paroxysms occur chiefly during the morning hours and that this fact is a diagnostic aid has been shown to be erroneous. The mean hour of occurrence of the temperature peak for the 1,000 paroxysms was 4:39 P. M. and that for rigorless paroxysms alone fell almost an hour later. Only 8.7 per cent of the observations occurred between midnight and 12 noon. It is true that some of the rigors exhibited a mean hour of onset shortly after noon. On the other hand the hour of onset of 319 rigors belonging to Group

IV in our series was 4:14 P. M. and that for all rigors was 3:00 P. M. Eighty-one per cent of the 654 rigors occurred during the afternoon hours.

That rigor indicates a more severe paroxysm and/or greater susceptibility of the patient is shown by the fact that only 51 per cent of the paroxysms of attacks less than three weeks in length were introduced by rigor, as compared with 71 per cent of paroxysms in longer attacks. The mean maximum observed temperature of the 654 rigoriferous paroxysms was 1.7° higher than that for 346 rigorless paroxysms, also a significant difference.

The reason for the later mean hour of occurrence of rigorless paroxysms (Table 6) is now apparent. The attack was ushered in by these paroxysms in the late afternoon, and by the time rigor was established the mean hour had anticipated to mid afternoon. Group IV paroxysms were an exception in that the mean hours of maximum temperature were identical for both types. There was a smaller proportion of rigorless paroxysms in this group and less anticipation among them. The subsequent rigoriferous ones occurred later in the day and tended to postpone. The combination of these circumstances resulted in the similar mean hours obtained.

Young (1944) found that the average length of the interval between febrile peaks of successive paroxysms of the same cycle was 43 hours and 25 minutes for the St. Elizabeth strain of *P. vivax,* and 45 hours and 46 minutes for a New Hebrides strain. He concluded, therefore, that the life cycle of these parasites was less than 48 hours. The corresponding mean interval for 900 paroxysms caused by our McCoy strain was found to be 47 hours and 44 minutes. We believe that such information merely indicates an overall, varying predominance of anticipation, and not only masks but does not explain the numerous occasions when several hours of postponement were noted.

The great majority of the rigorless paroxysms occurred during the first few days of the attack, the mean interval between first fever and first rigor being five days. Our data showed that by the eighth day rigor had been definitely established and that this day also marked the end of predominant anticipation for paroxysms of all groups. The daily mean maximum temperature reached its peak on the seventh day, when data for all groups were combined, while the mean maximum parasite density was attained on the ninth day. We believe that these events indicate a change of major importance in the host-parasite relationship at the end of the first week, signifying that the host has marshalled his forces to check the invasiveness of the parasites. For patients in Groups I and II this was definitely true; for those in Groups III and IV the effectiveness of the defense

mechanism was not so immediately apparent, although the shorter paroxysms during the latter half of these attacks may constitute evidence of the developing immunity.

Summary

This paper comprises the results of a study of the reactions exhibited by a series of 70 white patients to natural infection with a single strain of *Plasmodium vivax,* with particular attention paid to the febrile manifestations of the disease.

The 70 patients were divided into six groups; the attacks of patients in Groups I to IV varied from one to 63 days in length and have been utilized for detailed study of the febrile reactions, with supplementary data added from the infections of patients in Groups V and VI. Results may be summarized as follows:

1. The onset of vivax attacks may be characterized by a few days of continuous-remittent fever, or quotidian or tertian intermittent fever, or by a combination of both. Intermittency from the beginning is more apt to introduce shorter attacks and remittency the longer attacks. Regardless of type of onset, in our experience, most vivax attacks continue with quotidian periodicity.

2. The presence of rigor appeared to be an index of severity of the paroxysms: the proportion of paroxysms accompanied by rigor was 51.2 per cent for attacks up to three weeks in length and 71.1 per cent for longer attacks. An average of five days of clinical activity elapsed before the first rigor was observed. The mean parasite density on the day of first rigor was 4,012 per cubic millimeter, as compared with that of 274 for the day of first paroxysm. The mean duration for the 654 rigors was 52 minutes. Twenty-nine per cent of the rigors commenced with the patient's temperature at normal, and his temperature was less than 100° F. at the onset of 71.6 per cent of the rigors. The mean temperature at onset for the 654 rigors was 99.5°, and a mean increase in temperature of 4.2° was observed during rigor.

3. The mean maximum temperature observed was 104.2° for the 654 rigoriferous paroxysms, while that for the 346 rigorless paroxysms was 102.5°. The highest mean maximum temperature in the course of the 51 attacks was reached on the seventh day following onset.

4. With the maximum observed temperature serving as a marking point, two-thirds of the paroxysms occurred from 3 to 8 P. M. (inclusive), 17.3 per cent fell at 4 P. M., and only 8.7 per cent occurred from 1 A. M. to 12 M. Most inoculations were made in the morning, but in a series of 11 patients they were done from 8 to 9 P. M.; the mean hour of occurrence of maximum temperature of the paroxysms for the latter was 4:28 P. M. as compared with 4:39

P. M. for the others. The mean hour for paroxysms with rigor was 4:12 P. M., and that for the rigorless groups 5:31 P. M. The mean hour of onset for 654 rigors was 3:00 P. M.; that for the short attacks of Group I was 12:47 P. M., and that for the long attacks of Group IV was 4:14 P. M.

5. Paroxysms did not consistently occur at the same time as the predecessor of the same cycle throughout the attack. More paroxysms anticipated (40 per cent) than postponed (31 per cent), and the proportion of those which were isochronic (29 per cent) was least. Anticipation characterized rigorless paroxysms primarily and prevailed consistently over postponement only during the first week.

6. Attacks lasting up to three weeks were characterized by rigoriferous paroxysms (from onset to return of normal temperature), which were more than an hour longer than were those of the attacks of greater duration. The mean duration of paroxysms belonging to the first half of attacks lasting over three weeks was over two hours longer than that for the paroxysms of the second half; the difference lay altogether in the period of return from maximum to normal temperature. The patient's temperature at the close of rigor and the maximum attained varied directly to a significant degree with that at the onset of rigor.

7. The behavior of the two cycles characterizing quotidian activity, differentiated on the basis of onset following the day of inoculation, was similar with respect to all factors considered.

8. It is believed that a change occurred in the host-parasite relationship at about the end of the first week of the attack. Evidence of this was the establishment of rigor, the shift from anticipation to postponement in the hour of occurrence of paroxysms, and the attainment of the peak daily mean maximum temperature, followed by the crest of the parasitemia. The invasiveness of the parasites was apparently checked at this time, probably in response to the growing immunity of the host.

References

Coatney, G. R. and M. D. Young.
1942. A study of the paroxysms resulting from induced infections of *Plasmodium vivax*. Am. J. Hyg., 35:138-141.

James, S. P.
1926. Report on the first results of laboratory work on malaria in England. League of Nations Health Organization, Malaria Commission. (C.R./Malaria/57(1), p. 23.

Stratman - Thomas, W. K.
1941. The infection in the intermediate host. Symptomatology; vivax malaria, p. 184. A Symposium on Human Malaria. Washington, D. C. American Association for the Advancement of Science.

Young, M. D.
1944. Studies on the periodicity of induced *P. vivax*. J. Nat. Mal. Soc., 3:237-240.

NOTES ON THE PROLIFIC PRODUCTION AND DISPERSION OF *ANOPHELES QUADRIMACULATUS* FROM IMPOUNDED WATER BREEDING PLACES

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(Received for Publication 12 March 1945)

The generally accepted flight range of *Anopheles quadrimaculatus* for purposes of sanitary control is approximately one mile. This view has been well established, the original findings of LePrince and Griffitts (1917) having been substantiated by subsequent investigators. Smith, Watson and Crowell in 1940 presented a resume of the literature on the flight range of *A. Quadrimaculatus* and results of both entomological and malaria survey investigations relating to the flight range of *A. quadrimaculatus* in the Tennessee Valley area. Their observations indicated that while *A. quadrimaculatus* may fly a distance of a mile or more from a breeding place, its density is greatest within one-half mile of a breeding place, diminishing rapidly thereafter. Evidence was also presented to indicate that with low mosquito densities and fairly uniform population distribution approximately seventy-five per cent of malaria cases tend to occur within one-half mile of a breeding place, with little or no transmission of malaria beyond one mile. It was pointed out, however, that with high adult densities derived from prolific breeding areas, large numbers of *A. quadrimaculatus* may seek blood meals, and transmission of malaria may occur, at distances of more than a mile from a breeding place.

Under experimental conditions simulating massive production, Eyles and Bishop (1943) demonstrated a maximum flight range for *A. quadrimaculatus* of 2.5 miles. Clarke (1943) reported a single instance of flight of *A. quadrimaculatus* of approximately eight miles, and there are (Eyles, 1944) similar reports of flights up to three miles. These reports were based upon the finding of usually one or two mosquitoes at the stated distances from breeding places or point of release of stained specimen.

The partial filling of the Kentucky Reservoir on the lower Tennessee River during the late summer of 1944 provided a unique combination of conditions for observation of mass dispersion of *A. quadrimaculatus* under conditions of prolific propagation.

Closure of the Kentucky Dam was made 15 August 1944. The water rose from elevation 305′ to 343′ by 11 September, where it was held constant until 20 September and then raised to elevation 345′ on 22 September. Normal summertime pool elevation is

Table 1—Anopheline Larvae Densities in Kentucky Reservoir Based on Approximately 612 Square-Foot Dips

Date 1944	Area	Vegetation	Anopheles Larvae per Sq. Ft. Avg.	Max.	Identification % quad.	% punc.	Water Surface Temp. ° F.
6 Oct.	West Sandy	Hardwood (Oak, Hickory, etc.)	2.1	11	70	30	———
"	" "	Floating Hickory hulls & twigs	2.6	21	70	30	———
"	" "	*Salix nigra* (5 ft. seedlings)	3.9	6	40	60	———
10 Oct.	Paris Landing	*Fraxinus lanceolata*	19.6	32	100	0	80-85
"	" "	*Xanthium americanum*	6.8	13	100	0	"
"	" "	*Ambrosia trifida*	6.4	27	100	0	"
"	" "	*Echinochloa crus-galli*	7.6	13	100	0	"
"	" "	*Aster* sp.	6.3	11	100	0	"
"	" "	*Andropogon virginicus*	5.8	18	100	0	"
"	" "	*Ambrosia artemisiifolia*	1.5	5	100	0	"
"	" "	*Syntherisma sanguinale*	7.6	18	100	0	"
"	" "	*Lespedeza* sp.	10.7	30	100	0	"
"	West Sandy	Mixed Herbaceous	4.3	21	—	—	———
"	" "	Hardwood (Oak, Hickory, etc.)	2.3	11	—	—	———
11 Oct.	Paris Landing	*Echinochloa crus-galli*	15.6	52	86	14	———
17 Oct.	" "	*Echinochloa crus-galli*	49.9	100	100	0	72
"	" "	Mixed herbaceous and coppice	10.6	46	—	—	65-70
"	" "	Mixed herbaceous	4.1	18	—	—	60-70

Pest mosquitoes observed in abundance during the period of heavy quad production included *Psorophora ciliata, P. cyanescens, P. varipes, P. horrida, P. columbiae| Aedes vexans, Theobaldia inornata, Culex erraticus,* and *Uranotaenia sapphirina.*

Daytime biting rates of about 15 per hour were observed for *A. quadrimaculatus* in the woods above West Sandy Dike on 6 October 1944.

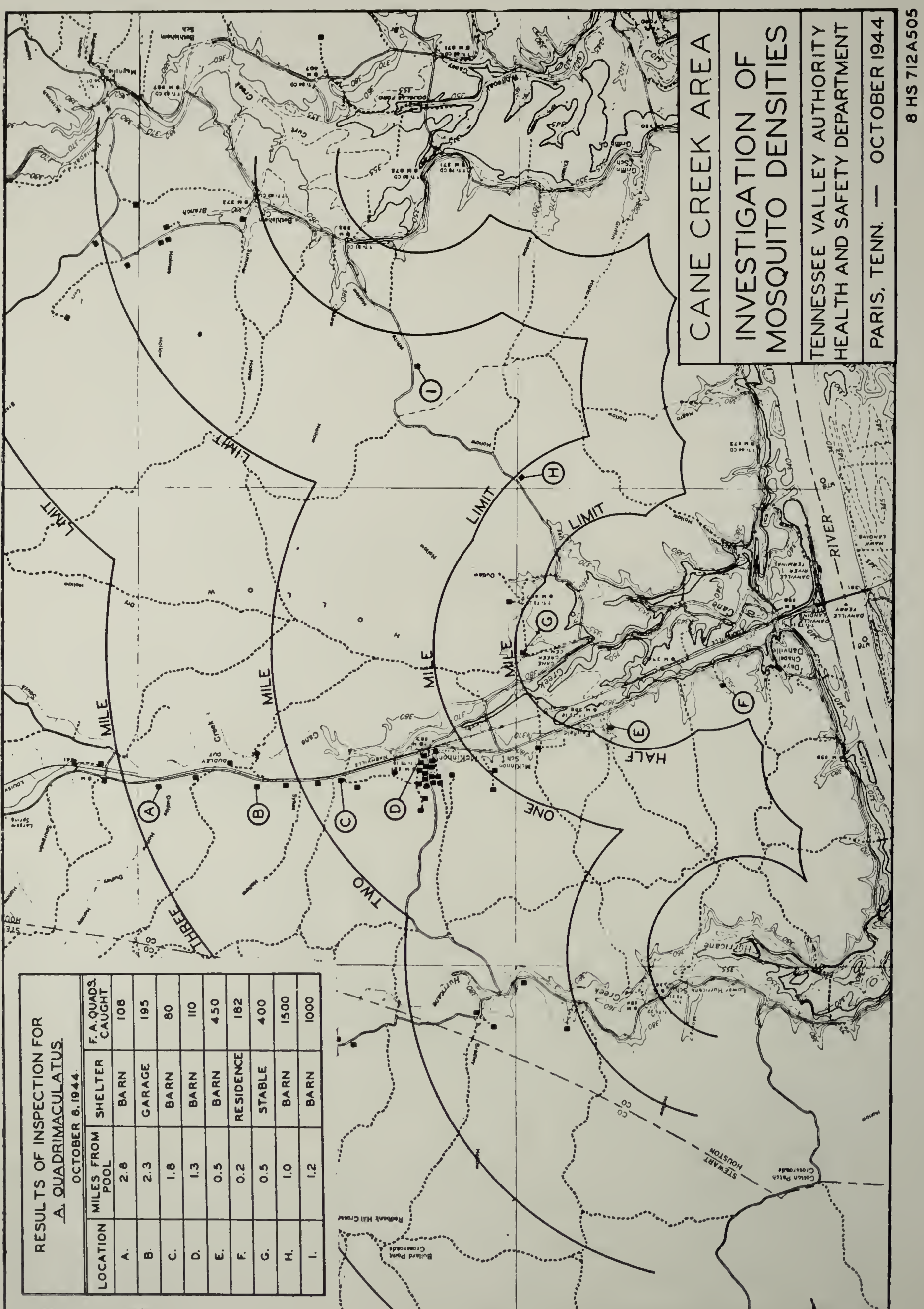
359′ with provisions for progressive recession to 354′ during the mosquito control season. Under the best of conditions, filling of a reservoir during the mosquito breeding season is undesirable from a malaria control standpoint. Because of the size of the Kentucky project (it is 184 miles long with 160,000 acres), it was not possible to prepare the basin for flooding during the summer months. Practically the entire basin was heavily grown up with annual growth, and coppice from preimpoundage clearing operations, and thousands of acres of anopheline breeding area were created as the broad flats were flooded to shallow depth around the margins of the impoundage.

By 15 September 1944 prolific production of *A. quadrimaculatus* had occurred and an inspection of fifteen adult mosquito collecting stations in the lower reservoir area gave an average female *A. quadrimaculatus* count of 136, with a maximum count at a single station of 500. During the following two weeks the average count rose to 272 and reached a maximum for the season of 480 during the week ending 7 October. Subsequently there was an abrupt fall in station counts coincident with the establishment of minimum atmospheric temperatures in the 30° to 50° F. range.

Records of larval densities in impounded water breeding places which were most probably the source of these adults are given in table 1. These data were collected after maximum adult densities were recorded. While they give a notion of the intensity of anopheline propagation during the last two weeks of September in these same situations, the actual larval densities during this time can only be surmised.

The high *A. quadrimaculatus* densities at the outer limits of the one-mile zone and numerous mosquito complaints of mosquito biting from residents living beyond the one-mile zone prompted an investigation to determine the approximate distance to which mass dispersion of *A. quadrimaculatus* was occurring from the partially filled lake. General observations had indicated that *A. quadrimaculatus* were ranging farthest from the lake up the broad flat valleys of tributary streams. Accordingly, one such valley, the Cane Creek area, was selected for investigation.

Cane Creek is a clear, rapidly flowing stream with gravel bottom and a minimum of pools and vegetation favorable for production of *A. quadrimaculatus*. Inspections had been made in this area over a five-year period as part of the preimpoundage studies on Kentucky Reservoir, and the highest number of *A. quadrimaculatus* found at any inspection during this time was three. The regular station in this area was usually negative, which indicates that prac-



RESULTS OF INSPECTION FOR A. QUADRIMACULATUS
OCTOBER 8, 1944.

LOCATION	MILES FROM POOL	SHELTER	F. A. QUADS. CAUGHT
A.	2.8	BARN	108
B.	2.3	GARAGE	195
C.	1.8	BARN	80
D.	1.3	BARN	110
E.	0.5	BARN	450
F.	0.2	RESIDENCE	182
G.	0.5	STABLE	400
H.	1.0	BARN	1500
I.	1.2	BARN	1000

tically no production of *A. quadrimaculatus* occurred in Cane Creek. above the backwater of the partially filled reservoir.

The extent and results of the investigation made in this area on 6 October 1944 are shown on a map of this portion of Kentucky Reservoir, with zone limits up to three miles from the partial impoundage. Counts of 105 and 195 female *A. quadrimaculatus* were recorded at distances of 2.8 and 2.3 miles respectively. The lower count of 80 at station C, 1.8 miles from the breeding area, was probably due to the fact that the barn had been sprayed with an insecticide approximately one hour before the inspection. A maximum count of 1,500 was recorded at station H which was approximately one mile from breeding areas on Cane Creek. Similarly, a count of 1,000 was found at station I which was approximately one mile from breeding areas on White Oak Creek. These exceptionally high counts at stations H and I were probably due in part to the lack of intervening sources of blood between the stations and the mosquito producing areas. From the map it will be noted that practically all of the houses in the Cane Creek area lie along the highway and railroad which were followed in making this investigation. With as many available sources of blood relatively close to the breeding area, the community of McKinnon being only 1.3 miles from the impoundage, it is likely that the mosquitoes found at the most distant points had probably taken one or more blood meals enroute; therefore, it is suggested that the distant dispersion noted was probably the result of one or more secondary flights and not the result of a single flight for an initial blood meal.

These data tend to substantiate further the opinions of many entomologists that, under conditions of unusually heavy production, significant numbers of *A. quadrimaculatus* may range considerably more than one mile from breeding areas. Since the observation was made toward the end of the mosquito breeding season, the long flight distances are suggestive of "prehibernation flights" similar to those reported by Freeborn (1932) for *A. freeborni.*

Summary and Conclusions

The partial filling of the Kentucky Reservoir in the late summer of 1944 resulted in prolific propagation of *A. quadrimaculatus.* An investigation in the Cane Creek tributary of this reservoir indicated that significant numbers of *A. quadrimaculatus* migrated approximately three miles from the impoundage. The conditions under which these long flight distances were observed suggest the possibility of "prehibernation flights" of *A. quadrimaculatus* and also the possibility that the distant dispersion of *A. quadrimaculatus*

may be the result of one or more secondary flights after an initial blood meal.

References

Clarke, J. Lyell
1943 Flight range and longevity of mosquitos dusted with aniline dye. Proceedings of the Thirtieth Annual Meeting of the New Jersey Mosquito Extermination Association, pages 227-235.

Eyles, Don E.
1944 A critical review of the literature relating to the flight and dispersion habits of anopheline mosquitoes. U. S. P. H. S. Bulletin No. 287, pp 23-25.

Eyles, Don E. and Lindsay K. Bishop
1943 An experiment on the range of dispersion of *Anopheles quadrimaculatus*. Am. J. Hyg., 37:239-245.

Freeborn, S. B.
1932 The seasonal life history of *Anopheles maculipennis* with reference to humidity requirements and hibernation. Am. J. Hyg., 16:215-223.

LePrince, J. A. and T. H. D. Griffitts
1917 Flights of mosquitoes. Studies on the distance of flight of *Anopheles quadrimaculatus*. Public Health Reports, 32:656-659.

Smith, Gordon E., Robert Briggs Watson and Robert L. Crowell
1940 Observations on the flight of *Anopheles quadrimaculatus*, Say. Am. J. Hyg., 34 (C): 102-113.

Postgraduate Course in Tropical Medicine Now Given at Tulane

A postgraduate course in Tropical Medicine for physicians is now in progress in the Department of Tropical Medicine of the Tulane School of Medicine, New Orleans. It extends from January through May, with full-time sessions throughout each day. The subject matter includes didatic, laboratory and clinical instruction in disease of warm climates, parasitic infections, medical protozoology, helminthology, entomology, mycology, public health problems, epidemiology, and control. In addition to the regular members of the departmental staff, eminent visiting specialists partake in the instruction. This year, the fifth that the course has been given, twenty-eight students are registered, representing several Central and South American countries, the West Indies, United States, India, Greece and missionary doctors who expect to practice in Africa, India and Honduras. Instruction in all aspects of malaria constitutes an important part of the course. It is expected that similar courses will be given yearly in the future.

THE RELATIONSHIP OF SPINAL FLUID TO PLASMA CONCENTRATIONS OF QUINACRINE AND QUININE

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(Received for publication 13 August 1945)

The mode of action and relative therapeutic efficiency of quinacrine and quinine in fulminating cerebral malaria have as yet not been conclusively demonstrated. An important factor may be the distribution of the drugs in the plasma, parenchyma of the brain, and spinal fluid. Lack of adequate information concerning the relationship of spinal fluid to plasma concentrations of these drugs made it desirable to investigate this phase of the problem.

Materials and Methods

In order to have a source of individuals from whom spinal fluids could be readily obtained, early or asymptomatic neurosyphilitic patients were used when being evaluated for future malarial therapy. The patients were divided into small groups so that a range could be obtained with reference to amounts of drugs given, periods of administration, and intervals at which spinal fluid and blood were withdrawn after the last dose of drug. Quinacrine was given as atabrine dihydrochloride dihydrate (1 gm. equivalent to 0.78 gm. base) and quinine as quinine sulfate dihydrate (1 gm. equivalent to 0.83 gm. base). The concentrations in this investigation are reported as base. In obtaining specimens the spinal fluid was withdrawn first and the venous blood immediately afterwards. The complete schedules are shown in Tables I and II. The concentration of quinacrine in plasma and spinal fluid was determined by the Brodie-Udenfriend method (1943) and the concentration of quinine by the following modification of the same method; the intensity of the fluorescent light obtained with the B^1-B^2 filter combination was compared with that obtained from a known amount of quinine added to plasma and carried through the entire procedure.

Quinacrine Results

In a previous study (Ellerbrook et al 1945) of the concentration of quinacrine in plasma in the treatment of vivax malaria of South Pacific origin, the reliability of the Brodie-Undenfriend method was investigated. When 0.25 microgram of quinacrine was added to 5 to 10 ml. of plasma the range of recovery was 88 - 106 per cent and with 1.0 microgram the range was 82 - 104

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Table 1—Simultaneous Plasma and Spinal Fluid Concentrations of Quinacrine

Patients In Group	*Amount of Drug Given Gms.*	*Dosage Schedule*	*Time Specimen Obtained After Last Dose (Hours)*	*Plasma Level Micrograms Per Liter*	*Spinal Fluid Level Micrograms Per Liter*
7	0.5	0.5 Gm. at 7AM	2-3	42	1
				43	0
				47	1
				47	3
				48	0
				50	0
				54	1
11	1.0	0.4 Gm. at 9AM 0.4 Gm. at 4PM 0.2 Gm. at 10PM	11-13	40	5
				41	6
				42	1
				46	1
				49	1
				52	0
				54	3
				55	3
				60	1
				64	1
				66	5
8	1.0	"	35-37	20	0
				22	3
				24	1
				25	0
				27	0
				27	1
				30	1
				45	1
5	2.0	As for 1 gm. total but on 2 successive days.	12-14	61	2
				75	1
				81	1
				87	1
				135	1
3	2.0	"	36-37	25	0
				52	1
				56	0
10		"	59-61	18	1
				43	2
				47	1
				51	2
				52	2
				55	3
				58	2
				61	1
				79	1
				80	2
7	3.0	As for 1 gm. total but on 3 successive days	35-37	32	1
				67	2
				80	2
				86	1
				100	2
				102	0
				128	4

per cent. The average recovery was approixmately 96 per cent. The reliability of the method when applied to spinal fluid was investigated in the current study by adding known small amounts of quinacrine to a total of 21 specimens of Wassermann negative, Wassermann positive, and pooled spinal fluids. No quinacrine was found in the spinal fluids themselves and when 0.080-0.250 microgram of quinacrine was added to 2 - 5 ml. specimens the maximum error of recovery was 0.02 micrograms. This maximum error in the 10 ml. specimens used for analysis corresponds to 2 micrograms per liter. The method was therefore considered suitable for the determination of concentration of quinacrine in spinal fluid of the same order of magnitude as those found in plasma. It is to be noted, however, that the maximum error of the method when applied to spinal fluid is approximately as great as the very small concentrations found.

In studying the relationship of the concentration of quinacrine in plasma to that in spinal fluid, the amounts of drug given and the periods of administration were selected in order to fulfill the following objectives: (1) Achievement of plasma concentrations at least as great as those usually obtained under adequate therapy for vivax malaria; (2) administration of still larger non-toxic doses; (3) variation of the rate of attainment of the concentrations and (4) variation of the length of time during which the concentration had been maintained before the specimens were taken. While it is true that such procedures may have no reference to the desirable dosage for fulminating cerebral malaria, the selections used appeared rational because they were safe and the range of concentrations maintained should presumably be adequate to demonstrate how freely quinacrine passes into spinal fluid.

The results of simultaneous plasma and spinal fluid levels are shown in Table I. It is apparent that in no instance was the spinal fluid concentration more than 15 per cent of the plasma value. Actually, in only 3 specimens were the cocentrations greater than 4 micrograms per liter, and only in 9 instances were the concentrations above 2 (the maximum error of the method.) In the 7 groups the amount of drug, period of administration and time of withdrawal of specimens after the last dose of drug did not appreciably alter either the spinal fluid concentrations found or their relationship to the plasma concentrations. The individual spinal fluid quinacrine concentrations obtained varied from 0-6 micrograms per liter while the average value for the 7 different groups varied from 0-2 micrograms per liter. The individual spinal fluid-plasma ratios obtained varied from 0-102 to 1-7.3 and the average ratios in the 6 treatment

groups varied from 1-132 to 1-20, but these values may not represent the true ratios because of the relatively larger error involved in determining the minute spinal fluid concentrations.

Table 2—Simultaneous Plasma and Spinal Fluid Concentrations of Quinine

Patients In Group	*Amount of Drug Given Gms.*	*Dosage Schedule*	*Time Specimen Obtained After Last Dose (Hours)*	*Plasma Level Micrograms Per Liter*	*Spinal Fluid Level Micrograms Per Liter*
8	0.47 Capsule	0.47 Gm. at 7AM	2.5-3.5	1290	19
				2310	39
				2310	67
				2320	43
				2410	43
				2420	40
				2890	21
				3040	46
6	0.94 Capsule	0.94 Gm. at 7AM	2.0-2.5	3040	109
				3510	145
				3630	95
				3860	90
				4150	104
				5010	113
7	1.00 Tablet	1.00 Gm. at 7AM	2.5-3.5	3060	84
				3360	102
				3480	82
				3510	48
				3900	89
				4970	130
				5830	120
15	1.41 Capsule	0.47 Gm. at 7AM 0.47 Gm. at 7PM 0.47 Gm. at 7AM	2.0-3.0	1360	41
				1780	45
				1950	57
				1990	60
				2040	183
				2290	45
				2690	99
				2840	66
				3210	122
				3270	96
				3950	171
				4180	156
				4440	106
				4630	135
				4740	93
4	2.35 Capsule	0.47 Gm. at 7AM 0.94 Gm. at 7PM 0.94 Gm. at 7AM	2.0-2.5	3910	155
				5130	230
				6050	230
				6820	250
4	3.00 Tablet	1.00 Gm. at 7AM 1.00 Gm. at 7PM 1.00 Gm. at 7AM	2.5-3.0	3320	200
				4790	240
				5740	260
				6740	250

Quinine Results

The accuracy of the method employed for determination of quinine is indicated by the following data: when 0.20-5.00 micrograms of quinine were added to 45 specimens of plasma and spinal fluid the quinine was recovered with an average error of 1 per cent (range −9 to +4 per cent). With the specimens containing 0.20-1.00 microgram (approximately the range encountered with the spinal fluid in this study) the maximum error in 16 of 18 specimens was 0.04 microgram which corresponds to a concentration of 4 micrograms per liter in the 10 ml. specimen usually employed. In two specimens the errors were 0.09 micrograms.

In studying the relationship of the concentration of quinine in plasma to that in spinal fluid an attempt was made to achieve plasma quinine concentrations of approximately the same order of magnitude as that obtained under adequate therapy for vivax malaria, to obtain some variation in the plasma concentrations, and to vary the time during which the drug was administered. The specimens were obtained at approximately the time the plasma concentrations had reached their peak values or shortly thereafter. In several groups the dosage schedules were so arranged that the amount of quinine base administered was identical with the amount of quinacrine base given to groups in the quinacrine study.

Simultaneous plasma and spinal fluid quinine concentrations are shown in Table II. In no instance was the spinal fluid concentration greater than 9 per cent of the corresponding plasma value while in only 3 specimens were the concentrations greater than 4 per cent of the plasma values. The average spinal fluid-plasma ratios in the 6 treatment groups varied from 1-58 to 1-20.

The average plasma quinine concentrations, spinal fluid quinine concentrations and spinal fluid-plasma ratios two to three and a half hours after administration of the last dose were as follows: after a single dose of 0.47 gm. the values were 2376, 40 and 1-58; after three doses of 0.47 gm. the values were 3020, 98 and 1-31; after a single dose of 0.94 gm. the values were 3870,109 and 1-36; after a dose of 0.94 gm. preceeded by 0.47 and 0.94 gm. the values were 4016, 94 and 1-43; after three doses of 1 gm. the values were 4016, 94 and 1-43; after three doses of 1 gm. the values were 5186, 238 and 1-22.

The plasma quinine concentrations found after a third dose of the drug were approximately one-third greater than the values obtained after a similar single dose and the average spinal fluid concentrations found were two to two and a half times as great as those obtained after a similar single dose.

The data indicate that the spinal fluid-plasma ratio tends to increased with an increase of the plasma quinine concentration and / or with the length of time over which the plasma concentration has been maintained.

Discussion

This study was undertaken primarily to determine the relationship of plasma to spinal fluid concentrations of quinacrine and quinine. Early or asymtomatic neurosyphilitic patients were used so that the data do not necessarily apply to normal individuals. In the case of quinacrine Shannon et al (1944) found in a study of two patients that the spinal fluid concentrations were 5 and 6 per cent respectively of the plasma concentrations. Such results were understandable on a basis of their studies of the distribution of quinacrine in blood in which it was shown that 80-90 per cent of the drug was bound to the non-diffusable constituents of plasma, presumably plasma albumin. Such binding left a maximum of about 20 per cent of the drug available for diffusion into spinal fluid. The mechanism of exchange of quinacrine between plasma and spinal fluid appears to be one of diffusion rather than secretion and our results are in accord with those of Shannon and associates. The explanation for the small percentage of quinine in spinal fluid as compared with plasma may also conceiveably be due to the existence of only a small fraction as unbound quinine in plasma water. The low concentrations of quinacrine in spinal fluid were not due to a lag in diffusion or distribution in the fluid since variation in the time of taking specimens after the last dose of drug and the period during which the plasma concentrations were maintained made no appreciable difference in results.

The concentrations in spinal fluid of quinacrine and quinine obtainable with non-toxic dosages probably cannot obtain sufficient magnitude to be of therapeutic value in cerebral malaria. However, it might be argued that a relatively small amount of either drug unbound in the spinal fluid water might conceivably act as a more effective agent than a larger amount bound to protein. It seems more likely that the concentrations in plasma and/or brain parenchyma are the important factors and it is probably the former since the parasites are located principally in the vessels of the brain rather than in the cells of the brain tissue.

Summary

1. Simultaneous plasma and spinal fluid drug concentrations were determined in 51 neurosyphilitic individuals given quinacrine and in 44 neurosyphilitic individuals given quinine.

2. The spinal fluid quinacrine concentrations varied from 0 to 6 micrograms per liter when the plasma concentrations ranged from 18 to 135 micrograms per liter. In only 9 out of 51 instances were the spinal fluid values above 2 micrograms per liter, the maximum error of the method.

3. The spinal fluid quinine concentrations varied from 19 to 260 micrograms per liter when the plasm aconcentrations ranged from 1290 to 6820 micrograms per liter. The spinal fluid plasma ratio tended to increase with an increase of the plasma quinine concentration and/or with the length of time over which the plasma concentration had been maintained.

4. In the different treatment groups the average spinal fluid-plasma ratios found were 1-132 to 1-20 with quinacrine and 1-50 to 1-20 with quinine.

References

Brodie, Bernard B. and Sidney Udenfriend
1943 The estimation of atabrine in biological fluids and tissues. J. Biolog. Chem., 151: 299-331.

Ellerbrook, L. D., S. W. Lippincott, C. F. Cateno, H. H. Gordon, and Alexander Marble.
1945. The concentration of atabrine in the treatment of *vivax* malaria acquired in the South Pacific. Unpublished data.

Shannon, James A., David P. Earle, Jr., Bernard B. Brodie, John V. Taggart, and Robert W. Berliner.
1944. The pharmacological basis for the rational use of atabrine in the treatment of malaria. Jour. Pharm. and Exper. Therapeutics, 81: 307-330:

NEW U.S.P.H.S. LABORATORY IN GEORGIA

The Malaria Investigations laboratory of the National Institute of Health, located at Moore General Hospital, Swannanoa, N. C., moved March 15th to the Milledgeville, Ga., State Hospital. At Swannanoa, investigations were made on the infectivity to American mosquitoes of asymptomatic foreign malarias in returned soldiers. At Milledgeville, investigations will be made upon the host-parasite relationships of native strains of plasmodia to both human and insect hosts. An effort will be made to delineate strains of native plasmodia and native mosquito vectors. The personnel will consist at present of P/A Sanitarian (R) Don E. Eyles, in Charge, and Laboratory Technicians Mary Nipper, Patricia Lee, and Marjorie Hollingsworth Clower.

NATIONAL MALARIA SOCIETY

Meeting conjointly with the Southern Medical Association

Minutes — 1945

Officers

Honorary President — Mr. J. A. LePrince, Memphis, Tennessee
President — Mr. H. A. Johnson, Memphis, Tennessee
President Elect — Dr. Mark F. Boyd, Tallahassee, Florida
Vice President — Clay G. Huff, Chicago, Illinois
Secretary-Treasurer — Dr. Mark F. Boyd, Tallahassee, Florida

Tuesday, November 13, 9 a. m.

The National Malaria Society convened for its twenty-eighth annual meeting in the Rockwood Room of the Sinton Hotel, Cincinnati, Ohio, at 9:00 a. m. on November 13, 1945, with Mr. H. A. Johnson, the president in the chair.

For this, the initial scientifc session, a program of thirteen contributed papers had been prepared. Of this number, however, three were, owing to the absence of their authors, presented by title. The meeting adjourned at 12:25 p. m.

The society reconvened in the Rockwood Room at 2:00 p. m. for a business session, and was called to order by the president.

A motion was adopted waiving reading of the minutes of the 1944 meeting and ordering their acceptance and approval as printed in the March 1945 (Volume IV, No. 1) issue of the society's journal.

The secretary-treasurer reported as follows:

From the 1944 roster of 394 active and 21 honorary members, two (Colonel W. N. Bispham, U.S.A. ret., and Mr. R. C. Shannon) have been lost by death, one by resignation, and 12 had been dropped for delinquency in dues. Forty-five new members had been gained by election. The current roster shows 424 active members of whom 343 are in good standing as of date of the report, and 21 honorary members. The treasury was reported to be in the following condition:

Balance of November 10, 1944	$3,640.44
Receipts from delinquent, current and advance dues, subscriptions, advertising and interest	2,814.37
	$6,454.81
Expenditures before paying for the 3rd and 4th issues of Volume IV of the Journal	1,578.35
Balance	$4,876.46

The report also listed assets of $5,251,46, and estimated liabilities of $1,092.40, leaving a net balance of $4,159.06 which is largely in the publication fund. The report of the secretary-treasurer was referred to a temporary auditing committee composed of Dr. Martin D. Young, Mr. John L. Porter and Mr. C. C. Kiker, for examination of the records and report.

On proceeding with regular business, the report of the committee on Medical Research was presented by Dr. G. Robert Coatney, and its reading was ordered by motion.* Thereafter a motion was presented directing the discontinuance of the annual reviews of scientific progress by the standing

* Being subseqently adopted by motion.

committees, which was tabled. A motion that a committee of five be appointed to prepare a report on terminology was likewise tabled.

Thereupon a motion was adopted directing that the regular order of business be suspended in order to receive the report of the special committee on the revision of the constitution and by-laws, consisting of Dr. E. L. Bishop, Dr. Trawick Stubbs and Mr. J. H. O'Neill. A motion was then adopted receiving the report of this committee, and directing that it be referred on the floor to the committee on resolutions. Thereupon a motion by Dr. F. J. Underwood, chairman of the resolutions committee, was adopted, calling for the immediate presentation of the report for discussion by the society in committee of the whole, and a further motion to take up consideration of the proposed draft section by section was adopted. Consideration was then given to the proposed constitution. Certain changes in the draft as submitted were made, some of minor degree by order of the president with general acquiescence of the assembly, others of a major character by motion. The most important changes directed: (a) omission and discontinuance of the classes of life and honorary memberships; (b) that the fiscal year co-incide with the calendar year: (c) that matters of dues be relegated to the by-laws; and (d) provision for an independent annual meeting in the event the Southern Medical Association should not meet.

Before adjournment, the secretary was directed to arrange a meeting place for an unscheduled business meeting to be held on the afternoon of the following day.

The meeting adjourned at 5:30 p. m.

Wednesday, November 14, 9 a. m.

The Society convened in the Roof Garden of the Gibson Hotel, in joint session with the American Society of Tropical Medicine, for consideration of a scientific program of twelve contributed papers, with Mr. J. H. Johnson and Doctor Rolla E. Dyer, presidents of the respective societies, jointly presiding. The meeting adjourned at 12:00

The society resumed consideration of business at 2:00 p. m., in parlor H of the Gibson Hotel, Mr. H. A. Johnson presiding. The Resolutions Committee was directed to make a presentation of the proposed by-laws. After free discussions from the floor, minor changes in the report were directed by the president with general acquiescence and major changes were directed by motions adopted. These principally affected: (a) deletion of provision for standing committees, excepting the Editorial Board, from the constitution and by-laws:. (b) authorizing a 10 per cent discount in subscriptions to the journal received in blocks of 10 or more; (c) deletion of time allotment for presentation of papers; and (d) authorizing the Editorial Board to enter into contractual or other arrangements for the printing of the Journal, subject to the approval of the proposed Board of Directors.

*Being subsequently adopted by motion.

The consideration of the report on revision of the constitution and by-laws having been completed, Doctor Underwood introduced the following resolution on behalf of the resolutions committee, which was on motion adopted, viz:

Whereas the report of the committee to study the constitution and by-laws had been considered, freely discussed and amended by the society during a two day session, the committee on resolutions recommends that the re-

port as revised be recommended to the society for final approval and adoption at the next annual meeting, and that copies of the amended report be distributed to the members prior to that time.

Doctor Underwood further introduced the following resolutions, which were on motion adopted, viz:

a) Expressing the thanks of the society to the special committee on the revision of the constitution and by-laws for their excellent work

b) Of sympathy and condolence to the widows of Colonel W. N. Bispham and Mr. R. C. Shannon, directing their transmission by the secretary-treasurer.

c) Of appreciation and thanks to Dr. Mark F. Boyd, retiring secretary-treasurer for his long and valued services in this office, and

d) Directing the secretary-treasurer to suitably express the appreciation and thanks of the society to the management of the Southern Medical Association and the Campbell-Kenton Medical Society for their hospitality and the facilities enjoyed; and to the management of the Sinton and Gibson Hotels for similar courtesies.

A motion was adopted directing that the reports of the standing committees, with the exception of that of the Editorial Board, be accepted and filed without reading. Reports were received from the following committees, viz: Engineering, Industrial Relations, Entomology, Epidemiology, and Statistics. The report of the Editorial Board was read by Doctor Watson, and was on motion accepted. A further motion was adopted, authorizing the payment of an honorarium of $25.00 to the editor's secretary for aid in preparation of the Journal.

Mr. Porter, for the auditing committee, reported that their examination of the report of the secretary-treasurer found it to be in accordance with his books, and introduced a motion for the acceptance of these reports, and recommended an honorarium of $100.00 to the secretary of the secretary-treasurer, which was adopted.

The secretary then presented a communication from the American Academy of Tropical Medicine, requesting that the society adopt a resolution petitioning the State Department to sponsor an International Congress on Tropical Medicine and Malaria at an early date in the United States, and that the president of the society appoint a member to serve on the organizing committee of such a congress. A motion was adopted supporting this proposal.

Mr. G. H. Bradley for the nominating committee, proposed the following slate of officers for 1945, viz:

Honorary President—Mr. J. A. LePrince, Memphis, Tennessee
President — Dr. Mark F. Boyd, Tallahassee, Florida
President Elect — Mr. Mark D. Hollis, Atlanta, Georgia
Vice President — Mr. J. A. Mulrennan, Jacksonville, Florida
Secretary-Treasurer — Dr. Martin D. Young, Columbia, S. C.

There being no nominations from the floor, a motion was adopted directing the secretary to cast the unanimous ballot of the society for the nominees.

There being no further business, the meeting adjourned *sine die* at 5:00 p. m.

Approved by Mr. H. A. Johnson, 26 November 1945.